



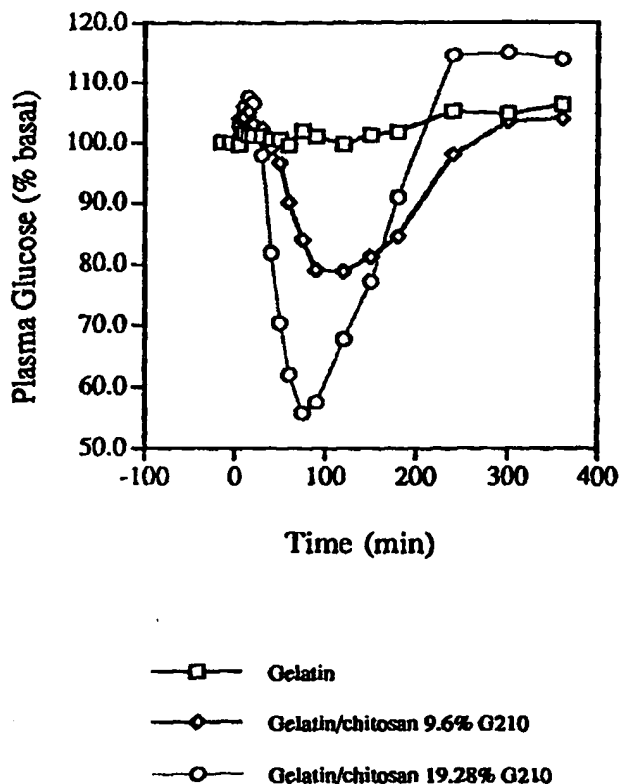
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 9/16, 9/00	A1	(11) International Publication Number: WO 98/30207 (43) International Publication Date: 16 July 1998 (16.07.98)
(21) International Application Number: PCT/GB98/00108 (22) International Filing Date: 14 January 1998 (14.01.98) (30) Priority Data: 9700624.1 14 January 1997 (14.01.97) GB (71) Applicant (for all designated States except US): DANBIOSYST UK LIMITED [GB/GB]; Albert Einstein Centre, Highfields Science Park, Nottingham NG7 2TN (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): WATTS, Peter, James [GB/GB]; Flat 2, 47 Highfield Road, West Bridgford, Nottingham NG2 6DR (GB). ILLUM, Lisbeth [DK/GB]; 19 Cavendish Crescent North, The Park, Nottingham NG7 1BA (GB). (74) Agent: BASSETT, Richard; Eric Potter Clarkson, St. Mary's Court, St. Mary's Gate, Nottingham NG1 1LE (GB).		(81) Designated States: AU, CA, GB, JP, NO, NZ, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>

(54) Title: CHITOSAN-GELATIN A MICROPARTICLES

(57) Abstract

There is provided a pharmaceutical composition for use in the improved uptake of therapeutic agents across mucosal surfaces which comprises a mixture of chitosan and a type A, cationic, gelatin, together with a therapeutic agent. The composition is preferably in the form of microparticles, such as microspheres.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LJ	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

CHITOSAN-GELATIN A MICROPARTICLES

This invention relates to novel drug delivery compositions which provide
5 for the improved uptake of therapeutic agents across mucosal surfaces.

Polar drugs, including high molecular weight peptides, proteins and polysaccharides, are typically not effectively absorbed across mucosal membranes, such as the gastrointestinal tract, the eye, the vagina, the
10 nasal cavity or the rectum. Such molecules are thus normally only given by injection, which inevitably gives rise to well known problems associated with patient compliance, the cost of treatment, as well as the potentially harmful effects, such as phlebitis and pain, of the injection.

15 It is well known in the literature that the absorption of polar molecules across mucosal membranes may be greatly improved if they are administered in combination with so-called "absorption enhancers". Examples of absorption enhancers which have been described in the literature include non-ionic surfactants, cyclodextrins, phospholipids and
20 bile salts. (For a review see Davis *et al* (eds.), *Delivery Systems for Peptide Drugs*, Plenum Press, New York, 1987; and Lee (ed.), *Peptide and Protein Delivery*, Marcel Dekker Inc., New York, 1991.)

EP-A-023 359 and EP-A-122 023 describe powdery pharmaceutical
25 compositions for application to the nasal mucosa, as well as methods for the administration of such compositions. The pharmaceutical compositions allow polypeptides and derivatives thereof to be effectively absorbed through the nasal mucosa. Similarly, US 4,226,849 describes a

method for administering a powdery medicament to the nasal mucosa, in which the preferred composition has mucoadhesive properties.

Formulations based on microspheres for mucosal delivery have been described in WO 88/09163. The formulations contain certain enhancers to aid effective penetration of the mucosa by the drug. WO 89/03207 describes microsphere formulations which do not require an enhancer.

Chitosan is a derivative of chitin or poly-N-acetyl-D-glucosamine in which the greater proportion of the N-acetyl groups have been removed through hydrolysis. It is available from several suppliers including Pronova, Drammen, Norway, and, depending on the grade selected, is soluble in water and/or aqueous acid up to pH values of between 6.0 and 7.0.

Chitosan has previously been used to precipitate proteinaceous material and to make surgical sutures. It has also been employed previously in oral drug formulations in order to improve the dissolution of poorly soluble drugs (see Sawayanagi *et al*, Chem. Pharm. Bull., 31 (1983) 2062-2068) or for the sustained release of drugs by a process of slow erosion from a hydrated compressed matrix (Nagai *et al*, Proc. Jt. US Jpn. Semin. Adv. Chitin Chitosan Relat. Enzymes, 21-39, Zikakis J.P. (ed.), Academic Press, Orlando, 1984).

WO 90/09780 describes a composition comprising a drug and a polycationic substance (e.g. chitosan) that promotes the transport of the drug across mucosal membranes. The composition may also comprise microspheres of the polycationic substance.

- WO 96/05810 describes a composition comprising a pharmacologically active compound and particles, preferably powders or microspheres, of chitosan or a chitosan derivative or salt, where the particles are either solidified or partially cross-linked such that they have a zeta-potential of between +0.5 and +50 mV. Solidified particles are made by treating particles made from a water soluble chitosan salt with an alkaline agent, such as sodium hydroxide, in non-acid containing water to render them insoluble.
- Chitosan microspheres have also been produced for use in enhanced chromatographic separation (Li Q. *et al*, Biomater. Artif. Cells Immobilization Biotechnology, 21 (1993) 391-398), for the topical delivery of drugs (Machida Y., Yakugaku Zasshi., 113 (1993) 356-368), for drug targeting after injection (Ohya Y *et al*, J. Microencap., 10 (1993) 1-9), as an implantable controlled release delivery system (Jameela and Jayakrishnan, Biomaterials, 16 (1995) 769-775) and for the controlled release of drugs (see Bodmeier R. *et al*, Pharm. Res., 6 (1989) 413-417 and Chithambara *et al*, J. Pharm. Pharmacol., 44, 1992, 283-286).
- EP 454044 and EP 486959 describe polyelectrolyte microparticles or polysaccharide microspheres, including chitosan microspheres, for use in the controlled release of drugs. Chitosan microspheres crosslinked with glutaraldehyde have also been described in JP 539149.
- Gelatin is a purified protein obtained either by partial acid hydrolysis (type A) or by partial alkaline hydrolysis (type B) of animal collagen. Type A gelatin is cationic with an isoelectric point between pH values of 7 and 9, whereas type B gelatin is anionic with an isoelectric point between pH values of 4.7 and 5. Gelatin is known to swell and soften when immersed

in cold water, eventually absorbing between 5 and 10 times its own weight in water. It is soluble in hot water, forming a gel on cooling. Gelatin is used as a haemostatic in surgical procedures as an absorbable film or sponge, which can absorb many times its own weight in blood. It is also
5 employed as a plasma substitute, and may be used in the preparation of pastes, pastilles, suppositories, tablets and hard and soft capsule shells for oral formulations.

The production of gelatin microspheres has been widely described in the
10 literature. Gelatin microspheres have been produced by an emulsification method involving crosslinking with glutaraldehyde, producing microspheres of less than 2 μm in diameter (Tabata and Ikada, Pharm. Res. 6 (1989) 422-427). Cortesi *et al* (Int. J. Pharm. 105 (1994) 181-186), Natruzzi *et al* (J. Microencapsulation, 11 (1994) 294-260) and
15 Esposito *et al* (Int. J. Pharm., 117 (1995) 151-158) have reported the production of microspheres of a mean diameter of 22 μm using a coacervation emulsification method. Microspheres as produced by the latter processes were not crosslinked. Microspheres of a smaller size have been produced according to a similar method by Esposito *et al* (Pharm.
20 Sci. Commun. 4 (1994) 239-246). The type of gelatin (A or B) used in these studies was not specified.

The production of microspheres by complexation, between a negatively charged material such as alginate and a positively charged chitosan has
25 been described in the literature. For example, Polk *et al*, J. Pharm. Sci., 83 (1994) 178-185) describes the production of chitosan-alginate microspheres by the addition of an alginate solution to a solution of chitosan and calcium ions. The highest concentration of chitosan used in the microsphere formulations was 5.2% w/w. Similarly, the formation of

complex coacervates between oppositely charged polyions, namely a positively charged chitosan and a negatively charged type B gelatin has been described by Remunan-Lopez and Bodmeier (Int. J. Pharm. 135 (1996) 63-72). These workers found the optimum chitosan:gelatin ratio to
5 be in the range 1:10 to 1:20. The coacervate was obtained in a dry form by decanting the supernatant after centrifugation and drying at 60°C.

We have now found, surprisingly, that microparticles, produced from a combination of a chitosan and a cationic type A gelatin, possess
10 particularly advantageous properties, which enable the improved transport of therapeutic agents, including polar drugs, across mucosal surfaces such as the nasal cavity.

Thus, according to a first aspect of the invention there is provided a
15 composition comprising a mixture of chitosan and type A, cationic, gelatin, together with a therapeutic agent (hereinafter referred to as "the compositions according to the invention").

By "mixture of chitosan and type A gelatin" we include any composition
20 comprising a chitosan, as defined hereinafter, and a type A gelatin, as defined hereinafter, whether a physical and/or chemical association between these two constituents exists or not.

The term "chitosan" will be understood by those skilled in the art to
25 include all derivatives of chitin, or poly-N-acetyl-D-glucosamine (including all polyglucosamine and oligomers of glucosamine materials of different molecular weights), in which the greater proportion of the N-acetyl groups have been removed through hydrolysis. We prefer that the chitosan has a positive charge.

Chitosan, chitosan derivatives or salts (e.g. nitrate, phosphate, sulphate, hydrochloride, glutamate, lactate or acetate salts) of chitosan may be used. We use the term chitosan derivatives to include ester, ether or
5 other derivatives formed by bonding of acyl and/or alkyl groups with OH groups, but not the NH₂ groups, of chitosan. Examples are O-alkyl ethers of chitosan and O-acyl esters of chitosan. Modified chitosans, particularly those conjugated to polyethylene glycol, are included in this definition. Low and medium viscosity chitosans (for example CL113, G210 and
10 CL110) may be obtained from various sources, including Pronova Biopolymer, Ltd., UK; Seigagaku America Inc., MD, USA; Meron (India) Pvt, Ltd., India; Vanson Ltd, VA, USA; and AMS Biotechnology Ltd., UK. Suitable derivatives include those which are disclosed in Roberts, *Chitin Chemistry*, MacMillan Press Ltd., London (1992).

15

The chitosan or chitosan derivative or salt used preferably has a molecular weight of 4,000 Dalton or more, preferably in the range 25,000 to 2,000,000 Dalton, and most preferably about 50,000 to 300,000 Dalton. Chitosans of different low molecular weights can be prepared by
20 enzymatic degradation of chitosan using chitosanase or by the addition of nitrous acid. Both procedures are well known to those skilled in the art and are described in recent publications (Li et al, (1995) Plant Physiol. Biochem. 33, 599-603; Allan and Peyron, (1995) Carbohydrate Research 277, 257-272; Damard and Cartier, (1989) Int. J. Biol. Macromol. 11,
25 297-302).

Preferably, the chitosan is water-soluble and may be produced from chitin by deacetylation to a degree of greater than 40%, preferably between 50% and 98%, and more preferably between 70% and 90%. Particular

deacetylated chitosans which may be mentioned include the "Sea Cure®" series of chitosan glutamates available from Protan Biopolymer A/S, Drammen, Norway.

- 5 The term "type A gelatin" includes all cationic proteins which are, or may be, obtained by partial acid hydrolysis of animal collagen, and excludes type B gelatins.

Although the compositions according to the invention may be prepared in
10 a variety of physical forms using techniques which will be well known to the skilled person, we prefer that the compositions are in the form of microparticles. The term "microparticles" includes microspheres, microcapsules and powders. However, we prefer that the microparticles are microspheres.

15

We have found, surprisingly, that when the compositions according to the invention are provided in the form of microparticles, such microparticles retain a positive charge and may provide for the improved transport of polar drugs across, or for the improved presentation of vaccines to,
20 mucosal surfaces, such as the nasal cavity, to such an extent that the effect is superior to that obtained for a chitosan solution, or microparticles produced from chitosan or type A gelatin alone (e.g. soluble (spray dried) chitosan microspheres and gelatin microspheres). The effect is also similar to that obtained for partially aldehyde crosslinked chitosan
25 microspheres, yet the compositions according to the invention are sufficiently hard/solid not to require crosslinking. We have further found that the flow properties of these chitosan/type A gelatin microparticles are superior to those of spray dried chitosan microspheres and crosslinked chitosan microspheres.

The microparticles may be prepared by spray drying, emulsification, solvent evaporation, precipitation or other methods known to a person skilled in the art. The therapeutic agent can be incorporated into the microparticles during their production or sorbed onto the microparticles after their production.

When the compositions according to the invention are in the form of microspheres, they may be prepared using for example either emulsification or spray drying techniques.

When microspheres are prepared by spray drying, a warm mixture of chitosan and type A gelatin is spray dried with instant cooling of the resultant microspheres. The therapeutic agent may be incorporated by adsorbing onto the surface of the microspheres by freeze drying or spray drying a suspension of the microspheres with the therapeutic agent, or by physically or mechanically mixing the dried microspheres with the therapeutic agent.

However, we have found that microspheres may advantageously be prepared by warming a solution of a chitosan mixed with type A gelatin, which is then emulsified and gelled by cooling. We have found that, in particular, microspheres prepared in accordance with this technique exhibit the advantageous properties referred to hereinbefore.

In the emulsification technique, the chitosan may be dissolved in water and mixed with type A gelatin under heating to 40°C causing the gelatin to melt. This mixture may be emulsified, at a temperature above the melting point of the gelatin, in an organic medium (e.g. a vegetable oil,

such as sunflower oil, soya oil, cotton seed oil or coconut oil), in the presence of an emulsifier with a low hydrophilic-lipophilic balance (HLB) value. Such emulsifiers, which are useful for stabilising water-in-oil emulsions, are known to those skilled in the art (e.g. Span 80). The
5 microspheres may then be solidified by decreasing the temperature of the emulsion to below 10°C with stirring. The microspheres may then be harvested using conventional techniques, for example by adding a pharmaceutically acceptable organic solvent, e.g. chilled acetone or petroleum ether, to the emulsion, centrifugation, washing and drying.
10 The therapeutic agent may be incorporated into the microspheres by adding it to the chitosan/gelatin mixture before emulsification. Alternatively, the therapeutic agent may be adsorbed onto the surface of the microspheres by freeze drying or by spray drying a suspension of the microspheres with the therapeutic agent, or by physically or mechanically
15 mixing the dried microspheres with the therapeutic agent.

Thus, according to a further aspect of the invention there is provided a drug delivery composition in a form suitable for administration to a mucosa comprising a therapeutic agent and microparticles made from a
20 mixture of chitosan and type A gelatin and where the agent is either incorporated into the particles during production or is adsorbed to the surface of the particles, or is present as an admixture.

Microcapsules and powders may be made by modifying the process as
25 defined herein in accordance with techniques which are well known to those skilled in the art, or may be prepared in accordance with other techniques which will be well known to those skilled in the art, including double emulsification processes.

According to a further aspect of the invention there is provided a process for the preparation of a composition according to the invention, which process comprises preparation of type A gelatin/chitosan microparticles (i.e. microparticles comprising a mixture of type A gelatin and chitosan) by a process of spray drying or by emulsification, which emulsification may comprise warming a solution of a chitosan mixed with type A gelatin, emulsification and gelation by cooling.

The flow properties of the microparticles can be measured by methods known to those skilled in the art. One possible method involves the measurement of the Hausner Ratio where a known weight of material is poured into a measuring cylinder and the volume recorded. The cylinder is then tapped against a surface a specified number of times and the volume again recorded. The poured and tapped densities are then determined and the Hausner Ratio = tapped density/poured density calculated. A ratio of < 1.25 indicates a free flowing material while a ratio of > 1.5 indicates a poor flowing (cohesive) material. Another possible method involves the measurement the Angle of Repose by pouring material through a funnel held at a fixed height onto a piece of graph paper until a cone is formed. The height (H) and the radius (R) of the cone is determined and the angle calculated ($\tan \theta = H/R$). An Angle of Repose $\theta < 30^\circ$ indicates good flow properties while an Angle of Repose $\theta > 40^\circ$ indicates very poor flow properties (James I. Wells, Pharmaceutical Preformulation, Ellis Horwood Series in Pharmaceutical Technology, 1988).

The size of the microparticles, which includes microcapsules and especially microspheres, is preferably in the range 1 to 200 μm , more

preferably 1 to 100 μm , as measured by e.g. light microscopy or sieve fractionation.

The microparticles will consist of preferably between 50 and 95%, more preferably between 70 and 90% and most preferably between 75 and 85% of type A gelatin, and correspondingly between 50 and 5%, preferably between 30 and 10% and most preferably between 25 and 15% of chitosan, as measured in relation to the total amount of gelatin and chitosan in the final composition (i.e. excluding therapeutic agent and other ingredients which may be included).

The term "therapeutic agent" includes drugs, genes (DNA) or gene constructs, vaccines and components thereof (for example isolated antigens or parts thereof) and monoclonal antibodies. For applications employing such materials as genes, gene constructs, vaccines and monoclonal antibodies, the microparticles can be used to enhance the delivery of the therapeutic agent into the mucosal tissue for enhanced therapeutic effect, for example presentation of an antigen to the underlying lymphoid tissue, and/or transfection of the cells in the mucosal lining.

Preferably the therapeutic agent is a polar drug. By "polar drugs" we mean molecules with a partition coefficient (octanol - water system) of less than 50.

The compositions may be used with therapeutic agents selected from the following non-exclusive list: insulin, PTH (parathyroid hormone), PTH analogues, PTHrP (human parathyroid hormone peptide), calcitonins (for example porcine, human, salmon, chicken or eel) and synthetic modifications thereof, enkephalins, LHRH (luteinising hormone releasing

hormone) and analogues (nafarelin, buserelin, leuprolide, goserelin), glucagon, TRH (thyrotropine releasing hormone), vasopressin, desmopressin, growth hormone, heparins, GHRH (growth hormone releasing hormone), CCK (cholecystokinin), THF (thymic humoral factor), CGRP (calcitonin gene related peptide), atrial natriuretic peptide, nifedipine, metoclopramide, ergotamine, pizotizin, pentamidine and vaccines (particularly but not limited to AIDS vaccines, measles vaccines, rhinovirus Type 13 and respiratory syncytial virus vaccines, influenza vaccines, pertussis vaccines, meningococcal vaccines, tetanus vaccines, diphtheria vaccines, cholera vaccines and DNA vaccines (e.g. one containing a plasmid DNA coding for a suitable antigen)).

Further therapeutic agents include but are not limited to: antibiotics and antimicrobial agents, such as tetracycline hydrochloride, leucomycin, penicillin, penicillin derivatives, erythromycin, sulphathiazole and nitrofurazone; anti-migraine compounds, such as naratriptan, sumatriptan, alnitidan or other 5-HT₁ agonists; vasoconstrictors, such as phenylephedrine hydrochloride, tetrahydrozoline hydrochloride, naphazoline nitrate, oxymetazoline hydrochloride and tramazoline hydrochloride; cardiotonics, such as digitalis and digoxin; vasodilators, such as nitroglycerine and papaverine hydrochloride; bone metabolism controlling agents, such as vitamin D and active vitamin D₃; sex hormones; hypotensives; anti-tumour agents; steroidal anti-inflammatory agents, such as hydrocortisone, prednisone, fluticasone, prednisolone, triamcinolone, triamcinolone acetonide, dexamethasone, betamethasone, beclomethasone and beclomethasone dipropionate; non-steroidal anti-inflammatory agents, such as acetaminophen, aspirin, aminopyrine, phenylbutazone, mefenamic acid, ibuprofen, diclofenac sodium, indomethacin, colchicine and probenecid; enzymatic anti-inflammatory

agents, such as chymotrypsin and bromelain seratiopeptidase; anti-histaminic agents, such as dephenhydramine hydrochloride, chloropheniramine maleate and clemastine; anti-tussive-expectorants, such as codeine phosphate and isoproterenol hydrochloride; analgesics, such as
5 opioids (like diamorphine, morphine and its polar metabolites, such as morphine-6-glucuronides and morphine-3-sulphate); anti-emetics, such as metoclopramide, ondansetron, chlorpromazine; drugs for treatment of epilepsy, such as clonazepam; drugs for treatment of sleeping disorders, such as melatonin; drugs for treatment of asthma, such as salbutamol.

10

Combinations of the abovementioned therapeutic agents may be employed.

The compositions according to the invention may be administered orally, nasally, vaginally, buccally, rectally, *via* the eye, or *via* the pulmonary
15 route, in a variety of pharmaceutically acceptable dosing forms, which will be familiar to those skilled in the art. For example, compositions may be administered *via* the nasal route as a powder using a nasal powder device, *via* the pulmonary route using a powder inhaler or metered dose inhaler, *via* the vaginal route as a powder using a powder device, formulated into a
20 vagina suppository or pessary or vaginal tablet or vaginal gel, *via* the buccal route formulated into a tablet or a buccal patch, *via* the rectal route formulated into suppositories; *via* the eye in the form of a powder or a dry ointment; and *via* the oral route in the form of a tablet, a capsule or a pellet (which compositions may administer agent *via* the stomach, the small
25 intestine or the colon), all of which may be formulated in accordance with techniques which are well known to those skilled in the art. The compositions may gel on the mucosa at least to some extent and this may facilitate retention of the composition on the mucosa.

The preferred route of administration is nasal. Devices which may be used to deliver the compositions according to the invention nasally include the Direct Haler®, the Bepak® powder device, the Monopoudre® (Valois) and the Insufflator® (Teijin).

5

Compositions according to the invention which may be administered orally may be adapted to deliver therapeutic agent to the small intestine or the colonic, especially the proximal colonic, region of the gastrointestinal tract.

- 10 Preferably, a means is provided to prevent release of therapeutic agent until the formulation reaches the small intestine or colon. Means which may be employed in order to prevent release until the small intestine is reached are well known to those skilled in the art (see for example dosage forms coated with so-called enteric polymers that do not dissolve in the acidic conditions
- 15 which exist in the stomach, but dissolve in the more alkaline conditions found in the small intestine of a mammal. Suitable enteric coating materials include modified cellulose polymers and acrylic polymers, and in particular those sold under the trademark Eudragit®.) Means which may be employed in order to prevent release until the colon is reached are well known to those
- 20 skilled in the art. Such materials include cellulose acetate trimellitate (CAT), hydroxypropylmethyl cellulose phthalate (HPMCP), polyvinyl acetate phthalate (PVAP), cellulose acetate phthalate (CAP) and shellac, as described by Healy in his article "Enteric Coatings and Delayed Release", Chapter 7 in *Drug Delivery to the Gastrointestinal Tract*, eds. Hardy *et al*,
- 25 Ellis Horwood, Chichester, 1989). Especially preferred materials are methylmethacrylates or copolymers of methacrylic acid and methylmethacrylate. Such materials are available as Eudragit® enteric polymers (Rohm Pharma, Darmstadt, Germany). Such a coating may also suitably comprise a material which is redox-sensitive (e.g. azopolymers

which may, for example, consist of a random copolymer of styrene and hydroxyethyl methacrylate, cross-linked with divinylazobenzene synthesised by free radical polymerisation, or disulphide polymers (see PCT/BE91/00006 and Van den Mooter, *Int. J. Pharm.* **87**, 37 (1992)). See
5 also International Patent Application WO 97/05903.

It will be appreciated by those skilled in the art that the site of delivery may also be selectively controlled by varying the thickness of certain of the abovementioned polymer coatings.

10

It will be well understood by those skilled in the art that further excipients may be employed in formulations comprising the compositions according to the invention. For example, in solid dosing forms, further excipients which may be employed include diluents such as microcrystalline cellulose (e.g.
15 Avicel®, FMC), lactose, dicalcium phosphate and starch(es); disintegrants such as microcrystalline cellulose, starch(es) and cross-linked carboxymethylcellulose; lubricants such as magnesium stearate and stearic acid; granulating agents such as povidone; and release modifiers such as hydroxypropyl methylcellulose and hydroxypropyl cellulose. Suitable
20 quantities of such excipients will depend upon the identity of the active ingredient(s) and the particular dosing form which is used.

If desired, other materials may be included in the composition, for example absorption enhancers. Suitable absorption enhancers include non-
25 ionic surfactants, cyclodextrins, bile salts and, preferably, phospholipids such as lysophosphatidylcholine, lysophosphatidylglycerol and generally those mentioned in WO 88/09163.

According to a further aspect of the invention, there is provided a pharmaceutical formulation in a form suitable for administration to a mucosal surface which comprises a composition according to the invention in a pharmaceutically acceptable dosage form.

5

Compositions according to the invention have been found to have the advantage that they provide improved transport of polar drugs across mucosal surfaces, such as the nasal cavity, have improved flow properties when compared to prior art compositions, and avoid the need for the use of chemical crosslinking agents.

10

According to a further aspect of the invention there is thus provided a method for the improved transport of therapeutic agents across (or into) mucosal surfaces (which includes the presentation of vaccines to mucosal surfaces) in mammals, and a method of treating a human or other mammal, which methods comprise administering a composition, as described above, preferably to a mucosal surface of that human or other mammal, for example the vagina, buccal cavity, rectum, lungs, eye, colon, small intestine, stomach or nasal cavity.

15

20

The amount of therapeutic agent which may be employed in the compositions according to the invention will depend upon the agent which is used. However, it will be clear to the skilled person that suitable doses of therapeutic agents can be readily determined non-inventively. Suitable doses are in the range 1 μ g to 1 g depending upon the therapeutic agent(s) which is/are employed and the route of administration.

25

The invention is illustrated, but in no way limited, by the following examples with reference to the figures in which:

Figure 1 shows the mean plasma glucose/time curves after administration to sheep of 2 IU/kg insulin in gelatin microspheres and in gelatin/chitosan microspheres containing either 9.6% or 19.28% G210 chitosan glutamate.

5

Figure 2 shows the mean plasma insulin/time curves after administration to sheep of 2 IU/kg insulin in gelatin microspheres and in gelatin/chitosan microspheres containing either 9.6% or 19.28% G210 chitosan glutamate.

10 Figure 3 shows the mean plasma calcium/time curves after administration to sheep of 20 IU/kg salmon calcitonin in gelatin microspheres and in gelatin/chitosan microspheres containing either 39.9% G110 or 19.9% G210 chitosan glutamate.

15 Figure 4 shows the mean plasma insulin/time curves after administration to sheep of 2 IU/kg insulin in 0.5% chitosan solution (G210) and in gelatin/chitosan microspheres containing 19.28% G210 chitosan glutamate.

20 Figure 5 shows the mean plasma insulin/time curves after administration to sheep of 2 IU/kg insulin with chitosan powder (G210) and in gelatin/chitosan microspheres containing 19.28% G210 chitosan glutamate.

25 Figure 6 shows the mean changes in plasma PTH concentration for a PTH/gelatin/chitosan microsphere formulation (PTH CHI/GER) as compared to a formulation comprising PTH (alone) in saline (PTH sol) and PTH with chitosan glutamate (PTH CHI Sol).

Figure 7 shows the effect on plasma glucose level of gelatin/chitosan microspheres comprising different amounts of insulin.

Figure 8 shows the effect of repeated administration of gelatin/chitosan
5 microspheres on plasma insulin level.

Example 1

Preparation of Microspheres Containing 3.6% w/w Insulin, 86.7% w/w Gelatin A and 9.6% w/w Chitosan Glutamate (Sea Cure G210)

10 193 mg chitosan glutamate was weighed into a 50 mL beaker and 15 mL of water was added and stirred until dissolution occurred. 1735 mg of gelatin A (Sigma) was added to the chitosan solution and stirred at 40°C until dissolution occurred. The pH of the solution was adjusted to 4 by adding an appropriate amount of 1M HCl. 72 mg of human zinc insulin
15 (1.8 mL of a 40 mg/mL insulin stock solution) was added to the gelatin/chitosan solution, which was transferred to a 20 mL volumetric flask and water added up to volume.

2 g of Span 80 was weighed into a metal beaker, 200 mL of sunflower oil
20 was added and the mixture warmed to 40°C. The 40°C insulin/gelatin/chitosan solution was added and emulsified at 1000 rpm for 5 minutes using a Heidolph stirrer fitted with a four blade stirrer arm maintaining the temperature at 40°C. The beaker was transferred to an ice bath and stirring continued at 1000 rpm until the temperature had
25 dropped to below 10°C. The stirring speed was reduced to 500 rpm, 150 mL of chilled acetone was added to the emulsion at 5 mL/min, and the mixture was then centrifuged at 2500 rpm in centrifuge tubes for 10 min. The supernatant was discarded and the pellet resuspended in 50 mL acetone. The microspheres were recovered by vacuum filtration and

washing with further 50 mL of chilled acetone. The filter cake was allowed to dry and the microspheres placed in 50 mL of acetone in a screw capped bottle containing a magnetic stirrer and stirred overnight. The microspheres were vacuum filtered and dried in a desiccator.

5

Example 2

Preparation of Microspheres Containing 3.6% w/w Insulin, 77.12% w/w gelatin A and 19.28% w/w Chitosan Glutamate (Sea Cure G210)

386 mg of chitosan glutamate was weighed into a 50 mL beaker, 15 mL of water was added and the resultant stirred until dissolution occurred. 1542 mg of gelatin A was added to the chitosan solution, which was then stirred at 40°C until dissolution occurred. The pH of the solution was adjusted to 4 by adding an appropriate amount of 1M HCl. 72 mg of human zinc insulin (1.8 mL of a 40 mg/mL insulin stock solution) was added to the gelatin/chitosan solution, which was then transferred to a 20 mL volumetric flask, and water was added up to volume.

2 g of Span 80 was weighed into a metal beaker, 200 mL of sunflower oil was added and the mixture warmed to 40°C. The 40°C insulin/gelatin/chitosan solution was added and emulsified at 1000 rpm for 5 minutes using a Heidolph stirrer fitted with a four blade stirrer arm maintaining the temperature at 40°C. The beaker was transferred to an ice bath and stirring continued at 1000 rpm until the temperature had dropped to below 10°C. The stirring speed was reduced to 500 rpm and 150 mL of chilled acetone was added to the emulsion at 5 mL/min which was then centrifuged at 2500 rpm in centrifuge tubes for 10 min. The supernatant was discarded and the pellet resuspended in 50 mL acetone. The microspheres were recovered by vacuum filtration and washed with further 50 mL of chilled acetone. The filter cake was allowed to dry, the

microspheres placed in 50 mL of acetone in a screw capped bottle containing a magnetic stirrer and stirred overnight. The microspheres were vacuum filtered and dried in a desiccator.

5 Example 3

Preparation of Microspheres Containing 0.2% w/w Salmon Calcitonin (SCT), 59.9% w/w Gelatin A and 39.9% w/w Chitosan Glutamate (Sea Cure G110)

798 mg chitosan glutamate (G110) was weighed into a 50 mL beaker, 15
10 mL of water was added and the resultant stirred until dissolution occurred. 1198 mg of gelatin A was added to the chitosan solution, which was then stirred at 40°C until dissolution occurred. The pH of the solution was adjusted to 4 by adding an appropriate amount of 1M HCl. 20,000 IU of SCT (0.91 mL of a 4 mg/mL SCT stock solution) was added to the
15 gelatin/chitosan solution which was transferred to a 20 mL volumetric flask, and water was added up to volume.

2 g of Span 80 was weighed into a metal beaker, 200 mL of sunflower oil was added and the mixture warmed to 40°C. The 40°C
20 SCT/gelatin/chitosan solution was added and emulsified at 1000 rpm for 5 minutes using a Heidolph stirrer fitted with a four blade stirrer arm maintaining the temperature at 40°C. The beaker was transferred to an ice bath and stirring continued at 1000 rpm until the temperature had dropped to below 10°C. The stirring speed was reduced to 500 rpm, 150
25 mL of chilled acetone was added to the emulsion at 5 mL/min which was then centrifuged at 2500 rpm in centrifuge tubes for 10 min. The supernatant was discarded and the pellet resuspended in 50 mL acetone. The microspheres were recovered by vacuum filtration and washed with a further 50 mL of chilled acetone. The filter cake was allowed to dry and

the microspheres placed in 50 mL of acetone in a screw capped bottle containing a magnetic stirrer and stirred overnight. The microspheres were vacuum filtered and dried in a desiccator.

5 Example 4.

Preparation of Microspheres Containing 0.2% w/w SCT, 79.9% w/w Gelatin A and 19.9% w/w Chitosan Glutamate (Sea Cure G210)

398 mg chitosan glutamate (G210) was weighed into a 50 mL beaker, 15 mL of water was added and the resultant mixture stirred until dissolution
10 occurred. 1598 mg of gelatin A was added to the chitosan solution, which was stirred at 40°C until dissolution occurred. The pH of the solution was adjusted to 4 by adding an appropriate amount of 1M HCl. 20,000 IU of SCT (0.91 mL of a 4 mg/mL SCT stock solution) was added to the gelatin/chitosan solution, which was then transferred to a 20 mL
15 volumetric flask and water was added up to volume.

2 g of Span 80 was weighed into a metal beaker, 200 mL of sunflower oil was added and the mixture was warmed to 40°C. The 40°C SCT/gelatin/chitosan solution was added and emulsified at 1000 rpm for 5
20 minutes using a Heidolph stirrer fitted with a four blade stirrer arm, maintaining the temperature at 40°C. The beaker was transferred to an ice bath and stirring continued at 1000 rpm until the temperature had dropped to below 10°C. The stirring speed was reduced to 500 rpm, 150 mL of chilled acetone was added to the emulsion at 5 mL/min which was
25 then centrifuged at 2500 rpm in centrifuge tubes for 10 min. The supernatant was discarded and the pellet resuspended in 50 mL acetone. The microspheres were recovered by vacuum filtration and washed with a further 50 mL of chilled acetone. The filter cake was allowed to dry and the microspheres placed in 50 mL of acetone in a screw capped bottle

containing a magnetic stirrer and stirred overnight. The microspheres were vacuum filtered and dried in a desiccator.

Example 5

- 5 The insulin-chitosan/gelatin microsphere formulations from Examples 1 and 2 were administered nasally to sheep and the effect of the formulations was compared to the effect of administering insulin in gelatin A microspheres.
- 10 The insulin - gelatin microspheres were prepared in the following way: 1928 mg gelatin A was added to 14 mL of water in a 50 mL beaker and heated under stirring at 40°C until the gelatin had dissolved. The pH of the gelatin solution was adjusted to 4 using 1M HCl and an equivalent of 72 mg of human zinc insulin (1.8 mL of 40 mg/mL insulin stock solution)
- 15 was added to the solution. The solution was transferred to a 20 mL volumetric flask and made up to volume. 2 g of Span 80 was weighed into a metal beaker, 200 mL of sunflower oil was added and the mixture warmed to 40°C. The 40°C insulin/gelatin solution was added and emulsified at 1000 rpm for 5 minutes using a Heidolph stirrer fitted with a
- 20 four blade stirrer arm, with the temperature maintained at 40°C. The beaker was transferred to an ice bath and stirring continued at 1000 rpm until the temperature had dropped to below 10°C. The stirring speed was reduced to 500 rpm and 150 mL of chilled acetone was added to the emulsion at 5 mL/min. The emulsion was centrifuged at 2500 rpm in
- 25 centrifuge tubes for 10 min., the supernatant discarded and the pellet resuspended in 50 mL acetone. The microspheres were recovered by vacuum filtration and washed with further 50 mL of chilled acetone. The filter cake was allowed to dry and the microspheres were placed in 50 mL of acetone in a screw capped bottle containing a magnetic stirrer and

stirred overnight. The microspheres were vacuum filtered and dried in a desiccator.

Each of the microsphere formulations were administered nasally to groups of five sheep using blue-line siliconised tubes at an insulin dose of 2 IU/kg and a microsphere dose of 2.0 mg/kg. Blood samples were collected at specified time points from the cannulated external jugular veins and plasma glucose and insulin concentrations measured. The mean changes in plasma glucose concentration with time for the three formulations are shown in Figure 1. It can be seen that insulin given nasally in combination with gelatin microspheres did not result in any significant lowering of the plasma glucose levels ($C_{\min} = 95.9\%$) whereas the formulations containing 9.6% chitosan and 19.28% chitosan gave glucose lowering effects of $C_{\min} = 74.6\%$ and $C_{\min} = 53.8\%$ of basal level, respectively. The corresponding plasma insulin levels for the three formulations are shown in Figure 2. It can be seen that C_{\max} for both the chitosan/gelatin microsphere formulations (131.6 mU/L and 439.7 mU/L for the 9.6/86.7% and 19.28/77.12% chitosan/gelatin microsphere, respectively) were significantly higher than the C_{\max} seen for the gelatin microspheres (53.5 mU/L).

Example 6

The calcitonin-chitosan/gelatin microsphere formulations described in Example 3 and 4 were administered nasally to sheep and the effect of the formulations compared to the effect of administering calcitonin in gelatin microspheres.

The calcitonin - gelatin microspheres were prepared in the following way: 1996 mg gelatin A was added to 15 mL of water in a 50 mL beaker and

heated under stirring at 40°C until the gelatin had dissolved. The pH of the gelatin solution was adjusted to 4 using 1M HCl and an equivalent of 20,000 IU of salmon calcitonin (0.91 mL of 4 mg/mL SCT stock solution) was added to the solution. The solution was transferred to a 20 mL volumetric flask and made up to volume. 2 g of Span 80 was weighed into a metal beaker, 200 mL of sunflower oil was added and the mixture warmed to 40°C.

The 40°C insulin/gelatin solution was added and emulsified at 1000 rpm for 5 minutes using a Heidolph stirrer fitted with a four blade stirrer arm, with the temperature maintained at 40°C. The beaker was transferred to an ice bath and stirring continued at 1000 rpm until the temperature had dropped to below 10°C. The stirring speed was reduced to 500 rpm and 150 mL of chilled acetone was added to the emulsion at 5 mL/min. The emulsion was centrifuged at 2500 rpm in centrifuge tubes for 10 min., the supernatant was discarded and the pellet resuspended in 50 mL acetone. The microspheres were recovered by vacuum filtration and washed with further 50 mL of chilled acetone. The filter cake was allowed to dry and the microspheres placed in 50 mL of acetone in a screw capped bottle containing a magnetic stirrer and stirred overnight. The microspheres were vacuum filtered and dried in a desiccator.

Each of the microsphere formulations were administered nasally to groups of five sheep using blue-line siliconised tubes at an SCT dose of 20 IU/kg and a microsphere dose of 2.004 mg/kg. Blood samples were collected at specified time points from the cannulated external jugular veins and plasma calcium concentrations measured. The mean changes in plasma calcium concentration with time for the three formulations are shown in Figure 3. It can be seen that SCT given nasally in combination with

gelatin microspheres only resulted in a minimal lowering of the plasma calcium levels ($C_{\min} = 91.1\%$) whereas the formulations containing 39.9% G110 chitosan and 19.9% G210 chitosan gave calcium lowering effects of $C_{\min} = 73.6\%$ and $C_{\min} = 74.7\%$ of basal level, respectively.

- 5 There was no significant difference between the effects obtained for the 39.9% G110 and 19.9% G210 chitosan levels in the gelatin microspheres.

Example 7

The insulin-chitosan/gelatin microsphere formulation described in
10 Example 2 was administered nasally to sheep and the effect of the formulation compared to the effect of administering insulin in a simple chitosan solution.

The microsphere formulation was administered nasally to a group of five
15 sheep using blue-line siliconised tubes at an insulin dose of 2 IU/kg and a microsphere dose of 2.0 mg/kg. As a comparison, a solution of 200 IU/mL insulin in 5 mg/mL G210 chitosan glutamate solution was administered nasally at 2 IU/kg to a group of four sheep. Blood samples were collected at specified time points from the cannulated external
20 jugular veins and plasma insulin concentrations measured. The mean changes in plasma insulin concentration with time for the two formulations are shown in Figure 4. It can be seen that the plasma insulin level is significantly higher for the gelatin/chitosan microsphere formulation ($C_{\max} = 450$ mU/L) as compared to the chitosan solution formulation ($C_{\max} =$
25 100 mU/L).

Example 8

The insulin-chitosan/gelatin microsphere formulation described in Example 2 was administered nasally to sheep and the effect of the

formulation compared to the effect of administering insulin with a chitosan powder formulation.

The gelatin/chitosan microsphere formulation was administered nasally to a group of five sheep using blue-line siliconised tubes at an insulin dose of 2 IU/kg and a microsphere dose of 2.0 mg/kg. As a comparison, a mixture of 640 IU insulin with 800 mg G210 chitosan glutamate was administered nasally at 2 IU/kg to a group of four sheep at 2 IU/kg. Blood samples were collected at specified time points from the cannulated external jugular veins and plasma insulin concentrations measured. The mean changes in plasma insulin concentration with time for the two formulations are shown in Figure 5. It can be seen that the plasma insulin level is significantly higher for the gelatin/chitosan microsphere formulation ($C_{\max} = 450$ mU/L) as compared to the chitosan powder formulation ($C_{\max} = 250$ mU/L). It should also be noted that the amount of chitosan administered in the two formulations is much higher for the chitosan powder formulation than for the gelatin/chitosan microsphere formulation.

Example 9

Preparation of Microspheres Containing 0.4% w/w PTH, 19.92% w/w Chitosan Glutamate (Sea Cure 210) and 79.68% w/w Gelatin A

9.28 mg of PTH was added to 20 mL of a solution containing 400 mg chitosan glutamate and 1.6 g of gelatin A and maintained at 50 - 60°C. 2 g of Span 80 was weighed into a beaker and 200 mL of soya oil was added. The resultant was mixed and heated to 40°C. The PTH/chitosan/gelatin solution was added and emulsified at 1000 rpm for 10 min. using a Heidolph stirrer fitted with a four blade stirrer arm, maintaining the temperature at 40°C. The beaker was transferred to an

27

ice bath and stirring continued at 100 rpm until the temperature had dropped to below 10°C. The stirring speed was reduced to 500 rpm and 150 mL of chilled acetone was added to the emulsion at 5 mL/min, followed by centrifugation at 3000 rpm for 10 min. The supernatant was discarded and the pellet resuspended in acetone. The microspheres were recovered by vacuum filtration and washed with 50 mL of chilled acetone. The filter cake was allowed to dry, the microspheres placed in 50 mL of acetone in a screw capped bottle containing a magnetic stirrer and stirred overnight. The microspheres were vacuum filtered and dried in a dissector.

Sheep study

The PTH gelatin/chitosan microsphere formulation was administered nasally to a group of 6 sheep using blue-line siliconised tubes at a PTH dose of 4 µg/kg. As a comparison, the same group of sheep was also administered the same dose of PTH in saline and in saline containing 0.5% chitosan glutamate. Blood samples were collected at specified time points from the cannulated external jugular veins and plasma PTH concentrations measured. The mean changes in plasma PTH concentration with time for the three formulations are shown in Figure 6. It can be seen that the plasma PTH is significantly higher for the gelatin in chitosan microsphere formulation ($C_{\max} = 2.5$ ng/mL) as compared to the chitosan solution formulation ($C_{\max} = 0.25$ ng/mL) and the control PTH solution ($C_{\max} = 0$ ng/mL).

25

Example 10Determination of Hausner Ratio for Chitosan/Gelatin A Microspheres and for Spray Dried Chitosan Microspheres (Sea Cure G210)

A known weight (see below) of chitosan/gelatin microspheres, prepared as in Example 2, was carefully poured into a 10 mL measuring cylinder and the volume recorded (poured volume). The measuring cylinder was tapped (onto the bench) 50 times and the volume of the chitosan/gelatin microspheres again recorded (tapped volume). The measurement was carried out in triplicate.

10

Weight	Poured Vol.(cm ³)	Poured Den.(g/cm ³)	Tapped Vol.(cm ³)	Tapped Den. (g/cm ³)	Hausner Ratio
2.2481	7.3	0.3079	5.8	0.3876	1.26
2.3680	7.6	0.3116	6.0	0.3947	1.27
15 2.3220	7.5	0.3096	6.1	0.3807	1.23

Hausner Ratio (chitosan/gelatin microspheres) = 1.25 (good flow properties)

20 A known weight (see below) of spray dried chitosan microparticles (Sea Cure G210; Pronova) was carefully poured into a 10 mL measuring cylinder and the volume recorded (poured volume). The measuring cylinder was tapped (onto the bench) 50 times and the volume of the chitosan again recorded (tapped volume). The measurement was carried
25 out in triplicate.

	Weight	Poured	Poured ²⁹	Tapped	Tapped	Hausner Ratio
		Vol.(cm ³)	Den.(g/cm ³)	Vol.(cm ³)	Den. (g/cm ³)	
	1.5609	9.0	0.1734	4.1	0.3807	2.20
	1.4728	8.5	0.1733	3.8	0.3876	2.24
5	1.4004	8.0	0.1751	3.6	0.3890	2.22

Hausner Ratio (chitosan) = 2.22 (very poor flow properties)

Example 11

10 Determination of Angle of Repose for Chitosan/Gelatin A Microspheres and for Spray Dried Chitosan Microspheres (Sea Cure G210)

The Angle of Repose (θ) was determined by pouring about 3 g of chitosan/gelatin microspheres, prepared as in Example 2, through a funnel (held at a fixed height) onto a piece of graph paper until a cone was
 15 formed. The height (H) and the radius (R) of the cone were determined and the Angle calculated ($\tan \theta = H/R$). The measurement was carried out in triplicate.

Mean Height = 10 mm

20 Mean Radius = 18 mm

Angle of Repose (chitosan/gelatin microspheres) = 29° (good flow properties)

The Angle of Repose (θ) was determined by pouring about 3 g of spray
 25 dried chitosan microparticles (Sea Cure G210; Pronova) through a funnel (held at a fixed height) onto a piece of graph paper until a cone was formed. The height (H) and the radius (R) of the cone were determined and the Angle calculated ($\tan \theta = H/R$). The measurement was carried out in triplicate.

Mean Height = 25 mm

Mean Radius = 24 mm

Angle of Repose (chitosan) = 46° (very poor flow properties).

5

Example 12

Determination of the Effect of Dose of Microspheres on the Absorption of Insulin

Microspheres were prepared as in Example 2 with the final concentration
10 of insulin in the microspheres being 2.0%, 4.0%, 5.3%, 7.7% and 14.4% w/w.

The microspheres were administered nasally to groups of 4 sheep with a fixed dose of 1 IU insulin/kg and 2.0, 1.0, 0.75, 0.5 and 0.25 mg/kg of
15 gelatin/chitosan microspheres as described in Example 8. The mean changes in plasma glucose level expressed as AUC are given in Figure 7. It can be seen that the effect of the gelatin/chitosan microspheres on the AUC is no different whether 2.0 mg/kg or down to 0.25 mg/kg of microspheres are administered with a constant dose of insulin.

20

Example 13

Effect of Repeated Administration of Gelatin A/Chitosan Microspheres on Plasma Insulin Level

Gelatin/chitosan/insulin microspheres were prepared as in Example 2.
25 The microspheres and a chitosan solution formulation, prepared as in Example 8, were administered nasally to groups of 4 sheep once daily for 5 consecutive days. A SC injection of insulin solution was given to the third group of sheep for five consecutive days. Plasma insulin levels expressed as AUC are given in Figure 8 . It can be seen that the AUC

obtained for each day is consistently higher for the gelatin/chitosan
microsphere formulation as compared to the chitosan solution formulation.
It can also be seen that the AUCs obtained on the five consecutive days
are similar for the nasal formulation, thus showing a consistent and
5 reproducible effect, whereas a certain accumulative effect can be seen for
the SC repeated injection.

Claims

1. A composition comprising a mixture of chitosan and type A, cationic,
5 gelatin together with a therapeutic agent.
2. A composition as claimed in Claim 1 wherein the composition is in the form of microparticles.
- 10 3. A composition as claimed in Claim 1 or Claim 2, wherein the therapeutic agent is incorporated into the particles during production, adsorbed to the surface of the particles, or is present as an admixture.
4. A composition as claimed in Claim 2 or Claim 3, wherein the
15 microparticles are microspheres.
5. A composition as claimed in any one of the preceding claims, wherein the composition is suitable for delivery of a therapeutic agent across a mucosal membrane into the systemic circulation.
- 20 6. A composition as claimed in any one of the preceding claims, wherein the chitosan has a molecular weight greater than 4000 Dalton.
7. A composition as claimed in Claim 6, wherein the chitosan has a
25 molecular weight in the range 25,000 to 2,000,000 Dalton.
8. A composition as claimed in Claim 7, wherein the chitosan has a molecular weight in the range 50,000 - 300,000 Dalton.

9. A composition as claimed in any one of the preceding claims, wherein the chitosan is a derivative, which derivative is formed by bonding of acyl or alkyl groups with the hydroxyl moieties of the chitosan.
- 5 10. A composition as in claimed in any one Claims 1 to 8, wherein the chitosan is in the form of a salt selected from the group nitrates, phosphates, sulphates, hydrochloride, glutamates, lactate or acetate.
- 10 11. A composition as claimed in any one of Claims 2 to 10, wherein the microparticles are produced by spray drying, emulsification, solvent evaporation or precipitation.
12. A composition as claimed in any one of the preceding claims, wherein the chitosan has a degree of deacetylation of greater than 40%.
- 15 13. A composition as claimed in Claim 12, wherein the degree of deacetylation is between 50 and 98%.
14. A composition as claimed in Claim 13, wherein the degree of deacetylation is between 70 and 90%.
- 20 15. A composition as claimed in any one of Claims 2 to 14 wherein the microparticles have a diameter of between 1 - 200 μm .
- 25 16. A composition as claimed in Claim 15, wherein the diameter is between 1- 100 μm .
17. A composition as claimed in any of the preceding claims, wherein the composition comprises between 50 and 95% of type A gelatin.

18. A composition as claimed in Claim 17, wherein the composition comprises between 75 and 85% of type A gelatin.

5 19. A composition as claimed in any one of the preceding claims wherein the therapeutic agent is a polar drug.

20. A composition as claimed in any one of the preceding claims, wherein the therapeutic agent is a polypeptide.

10

21. A composition as claimed in Claim 20, wherein the therapeutic agent is selected from the group insulin, calcitonin, luteinising hormone releasing hormone, growth hormone or a growth hormone releasing factor.

15

22. A composition as claimed in any one of Claims 1 to 19, wherein the therapeutic agent is an analgesic agent or a drug for the treatment of migraine.

20 23. A composition as claimed in any one of Claims 1 to 19, wherein the therapeutic agent is an antigen intended for mucosal immunisation.

24. A composition as claimed in any one of Claims 1 to 19, wherein the therapeutic agent is a gene or gene construct (DNA) intended for the
25 transfection of cells in the mucosal surface.

25. A composition as claimed in any one of the preceding claims, which further comprises an absorption enhancing agent.

26. A composition as claimed in Claim 25 where the absorption enhancing agent is a phospholipid.

27. A pharmaceutical formulation in a form suitable for administration to a mucosal surface which comprises a composition as defined in any one of the preceding claims, in a pharmaceutically acceptable dosage form.

28. A formulation as claimed in Claim 27, wherein the mucosal surface is in the nose.

29. A formulation as claimed in Claim 27, wherein the mucosal surface is in buccal cavity.

30. A formulation as claimed in Claim 27, wherein the mucosal surface is in the vagina.

31. A formulation as claimed in Claim 27, wherein the mucosal surface is in the gastrointestinal tract and the formulation is administered *via* the mouth.

32. A formulation as claimed in Claim 27, wherein the mucosal surface is in the rectum.

33. A formulation as claimed in Claim 27, wherein the mucosal surface is in the eye.

34. A formulation as claimed in Claim 27, wherein the mucosal surface is in the lung.

36

35. A method for the improved transport of therapeutic agents across mucosal surfaces in mammals, which comprises administering a composition, as defined in any one of Claims 1 to 26, or a formulation as defined in any one of Claims 27 to 34, to a mucosal surface of said
5 mammal.

36. A method of treating a mammalian patient which comprises administering a composition, as defined in any one of Claims 1 to 26, or a formulation as defined in any one of Claims 27 to 34, to such a patient.

10

37. A method as claimed in Claim 36 wherein the composition or formulation is delivered to a mucosal surface.

38. A method as claimed in any one of Claims 35 to 37, wherein the
15 composition or formulation is adapted to deliver a therapeutic agent across a mucosal membrane into the systemic circulation.

39. The preparation of type A gelatin/chitosan microparticles by a process of spray drying.

20

40. The preparation as claimed in Claim 39, characterised in that the microparticles are prepared by spray drying a warm mixture of chitosan and gelatin with instant cooling of the resultant microparticles.

25 41. The preparation of type A gelatin/chitosan microparticles by emulsification.

42. The preparation as claimed in Claim 41, characterised in that the microparticles are prepared by warming a solution of a chitosan mixed with type A gelatin, emulsification and gelation by cooling.

5 43. The preparation as claimed in Claim 42, characterised in that the chitosan is dissolved in water and mixed with the gelatin under heating to 40°C.

44. The preparation as claimed in Claim 42 or Claim 43, characterised in
10 that the mixture is emulsified at a temperature above the melting point of the gelatin in an organic medium, in the presence of an emulsifier.

45. The preparation as claimed in any one of Claims 42 to 44,
characterised in that the microparticles are solidified by decreasing the
15 temperature of the emulsion below 10°C.

46. The preparation as claimed in any one of Claims 42 to 45,
characterised in that the microparticles are harvested by adding chilled
acetone to the emulsion, centrifugation, washing and drying.

20

47. The preparation as claimed in any one of Claims 41 to 46,
characterised in that the microparticles further comprise a therapeutic
agent, which agent is incorporated into the microparticles by adding it to
the mixture before emulsification.

25

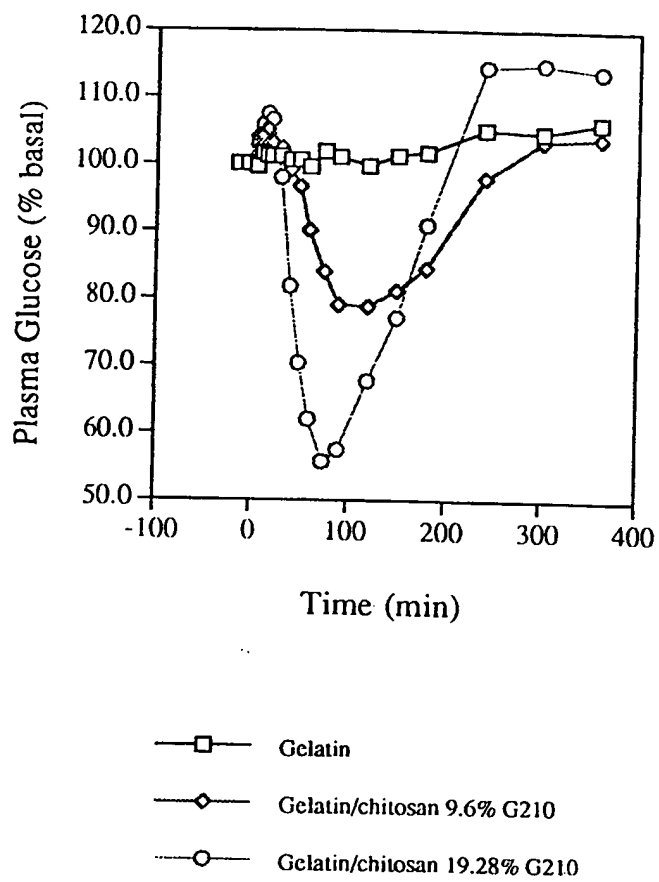
48. The preparation as claimed in any one of Claims 39 to 46,
characterised in that the microparticles further comprise a therapeutic
agent, which agent is adsorbed onto the surface of the microparticles by

freeze drying or spray drying a suspension of microparticles with the therapeutic agent.

49. The preparation as claimed in any one of Claims 39 to 46,
5 characterised in that the microparticles further comprise a therapeutic agent, which agent is adsorbed onto the surface of the microparticles by physically or mechanically mixing the dried microparticles with the therapeutic agent.
- 10 50. The use of a composition, as defined in any one of Claims 1 to 26, or a formulation as defined in any one of Claims 27 to 34, in the manufacture of a medicament for use in the improved transport of therapeutic agents across mucosal surfaces in mammals.

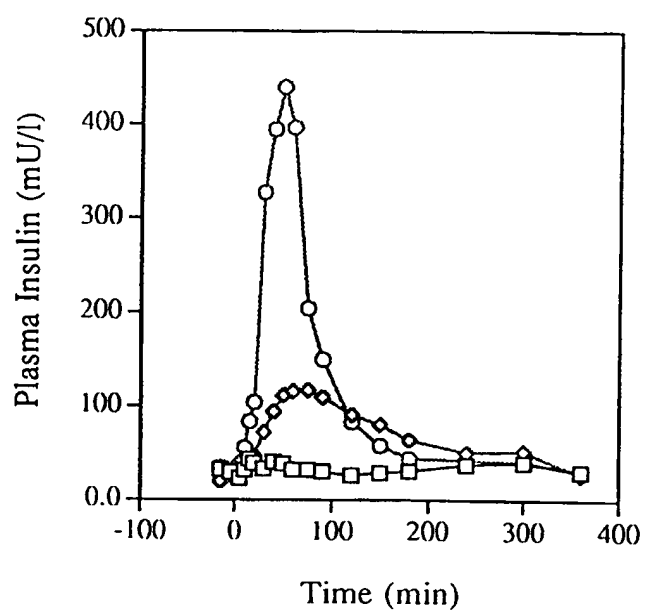
1/8

Figure 1



2/8

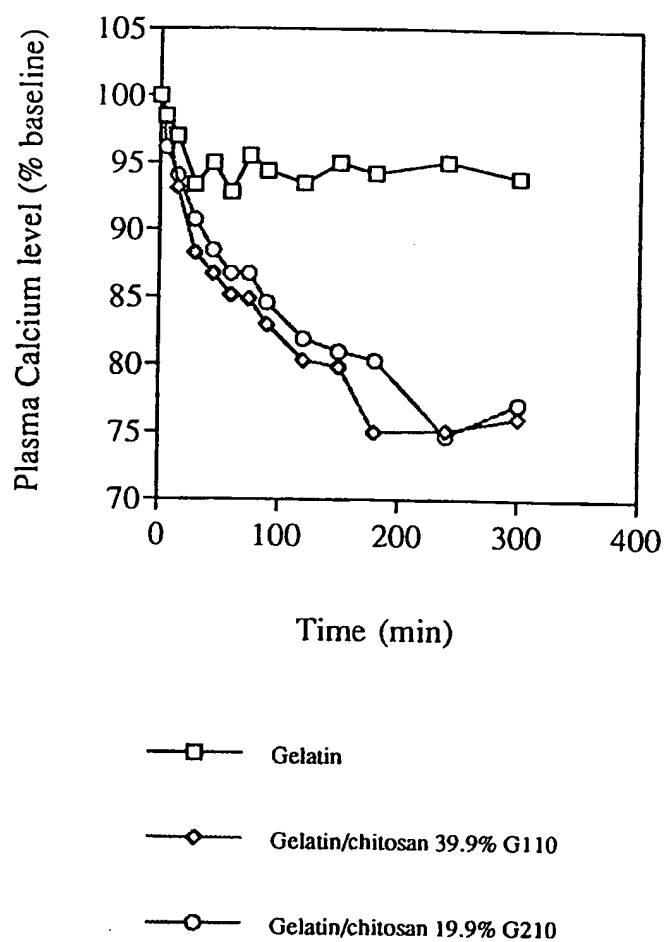
Figure 2



- Gelatin
- ◇— Gelatin/chitosan 9.6% G210
- Gelatin/chitosan 19.28% G210

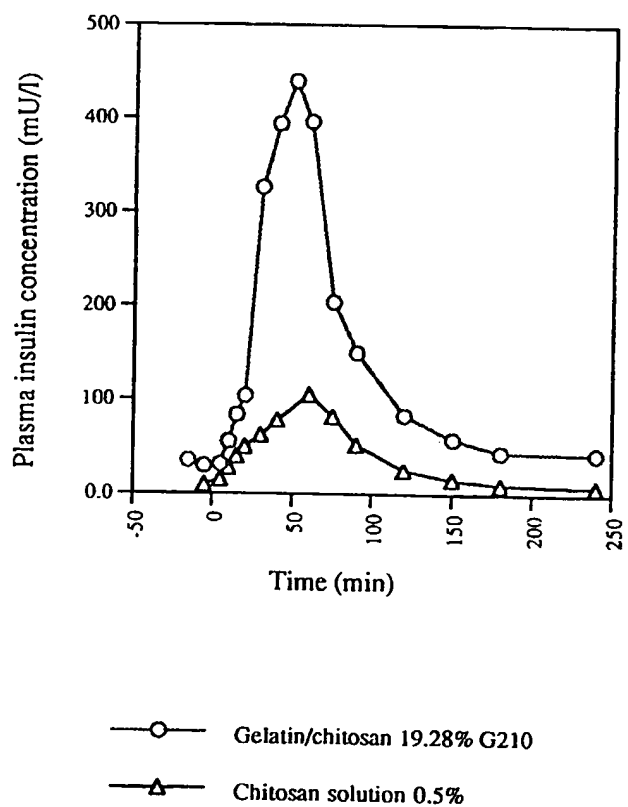
3/8

Figure 3



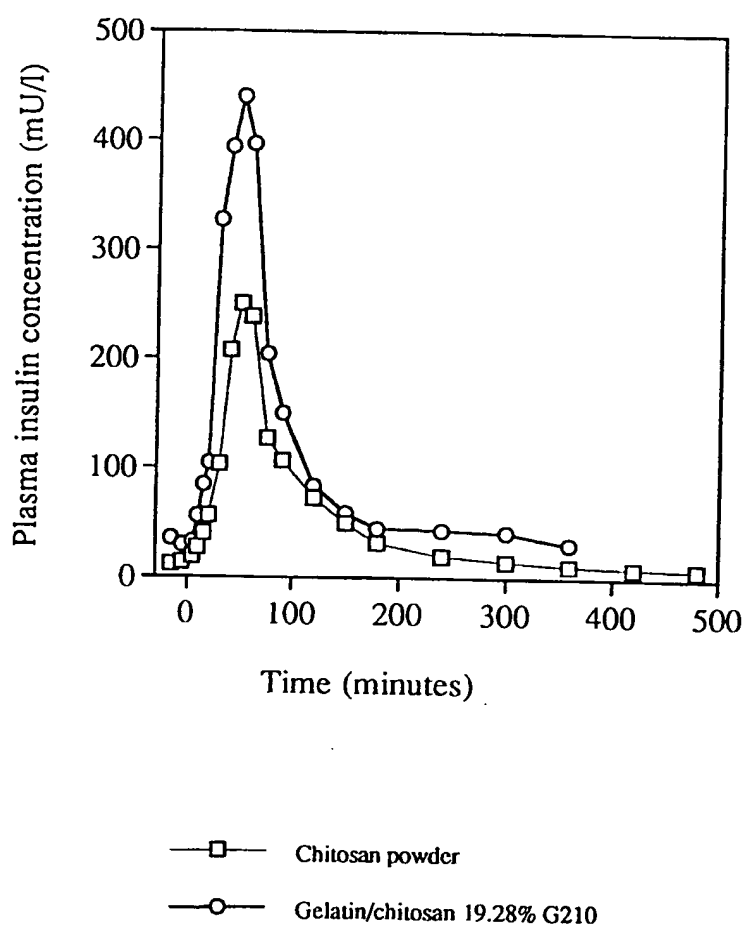
4/8

Figure 4



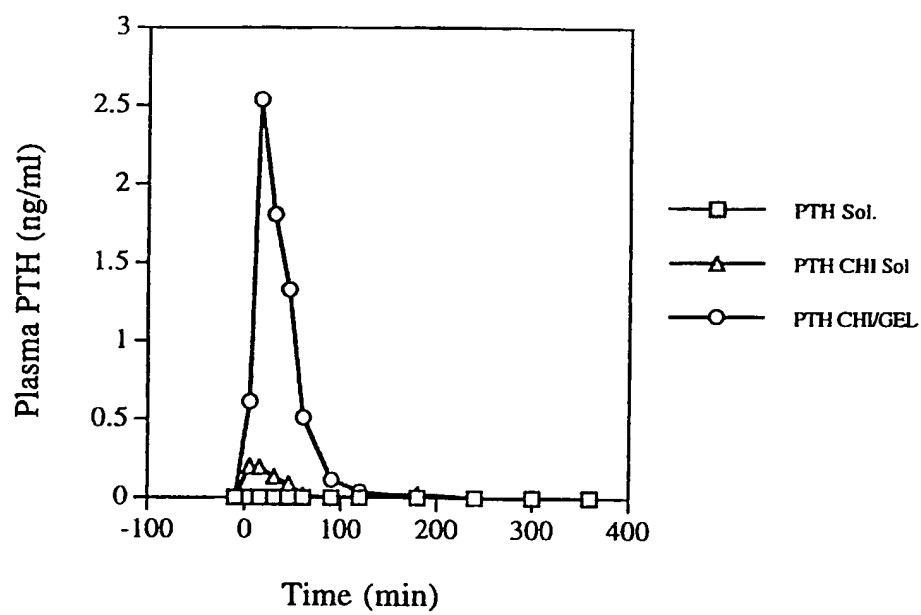
5/8

Figure 5



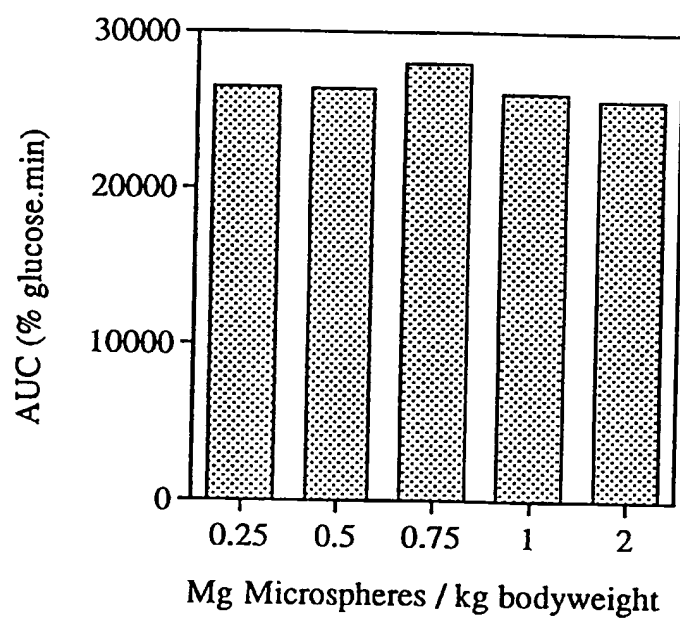
6/8

Figure 6



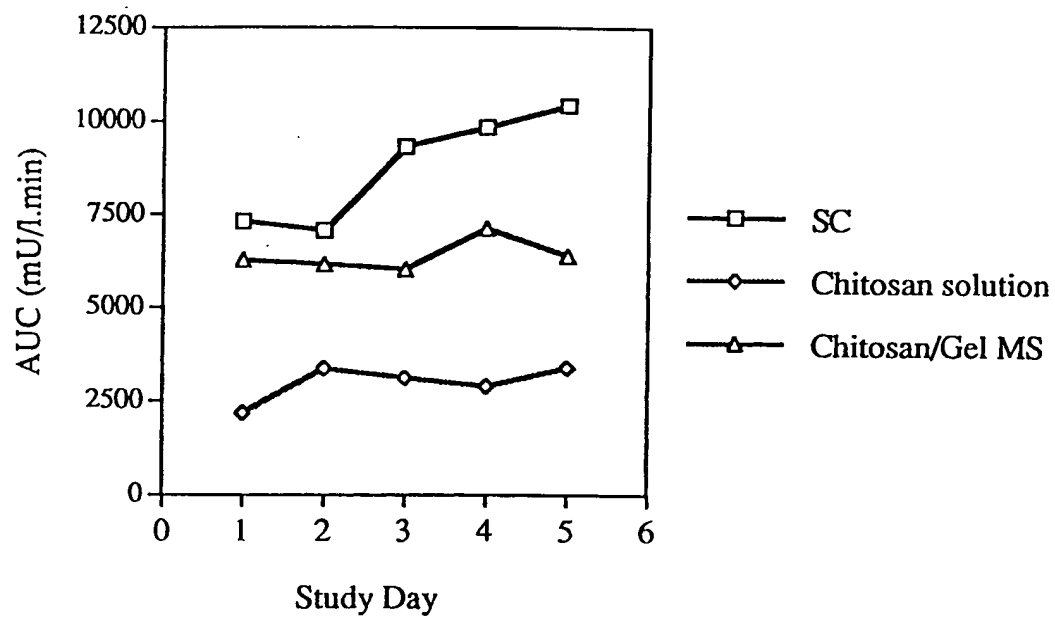
7/8

Figure 7



8/8

Figure 8



INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/00108

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/16 A61K9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 470 872 A (ALKO LTD.) 12 February 1992 see claims 1,5,12,13 ---	1-50
A	EP 0 376 385 A (THE PROCTER & GAMBLE) 4 July 1990 see claims 1,2 ---	1-50
A	CARMEN REMUÑAN LOPEZ, ET AL.: "Effect of formulation and process variables on the formation of chitosan-gelatin coacervates" INTERNATIONAL JOURNAL OF PHARMACEUTICS, vol. 135, no. 1,2, 1996, pages 63-72, XP002059231 cited in the application see the whole document ---	1-50
-/--		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

17 March 1998

Date of mailing of the international search report

27/03/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Ventura Amat, A

INTERNATIONAL SEARCH REPORT

Internat. Application No

PCT/GB 98/00108

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WO 96 03142 A (DANBIOSYST UK LIMITED) 8 February 1996 see the whole document -----</p>	1-50

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 98/00108

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 470872 A	12-02-92	AT 112700 T	15-10-94
		AU 642033 B	07-10-93
		AU 8174491 A	13-02-92
		CA 2042529 A	11-02-92
		DE 69104576 D	17-11-94
		DE 69104576 T	16-03-95
		ES 2065624 T	16-02-95
		JP 4281836 A	07-10-92
		NZ 239349 A	25-03-94
		US 5543162 A	06-08-96
EP 376385 A	04-07-90	AU 4732389 A	05-07-90
		CA 2004270 A	29-06-90
		CN 1044295 A	01-08-90
		DK 672689 A	30-06-90
		JP 2258900 A	19-10-90
WO 9603142 A	08-02-96	AU 2988695 A	22-02-96
		CA 2195639 A	08-02-96
		EP 0773791 A	21-05-97
		FI 970331 A	27-01-97
		GB 2305606 A	16-04-97
		NO 970252 A	21-01-97



LRIF. D I

Am

Numéro de publication: **EP0952822***

(WO9830207)

A1

Date de publication : **03.11.1999**

N° de dépôt : **EP98900600.2**

Date de dépôt : **14.01.1998**

N° de priorité : **GB9700624**

Date de priorité : **14.01.1997**

Indice principal CIB : **A61K-009/16**

Indice secondaire CIB : **A61K-009/00**

Déposant(s) : **DANBIOSYST UK LIMITED**

Inventeur(s) : **WATTS, Peter, James, ILLUM, Lisbeth**

MICROPARTICULES A BASE DE CHITOSANE ET DE GELATINE DE TYPE A

Voir le résumé dans la demande internationale PCT correspondante : **WO9830207**

Etats désignés : **AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE**

[View Images \(51 pages\)](#) | [View Cart](#) | [View INPADOC only](#)

Add to cart: [PDF \(-4850 KB\)](#) | [TIFF](#) | [Fax](#) | [File History](#) | [More choices...](#)

Country: **WO** World Intellectual Property Organization (WIPO)

Kind: **A1** Publ.OF the Int.Appl. with Int.Search Report

Inventor(s): **WATTS, Peter, James** , Flat 2, 47 Highfield Road, West Bridgford, Nottingham NG2 6DR, United Kingdom
ILLUM, Lisbeth , 19 Cavendish Crescent North, The Park, Nottingham NG7 1BA, Denmark

Applicant(s): **DANBIOSYST UK LIMITED**, Albert Einstein Centre, Highfields Science Park, Nottingham NG7 2TN, United Kingdom
[News, Profiles, Stocks and More about this company](#)

Issued/Filed Dates: **July 16, 1998 / Jan. 14, 1998**

Application Number: **WO1998GB0000108**

IPC Class: **A61K 9/16; A61K 9/00;**

ECLA Code: **A61K9/16H6F; A61K9/00M14; A61K9/16H6H;**

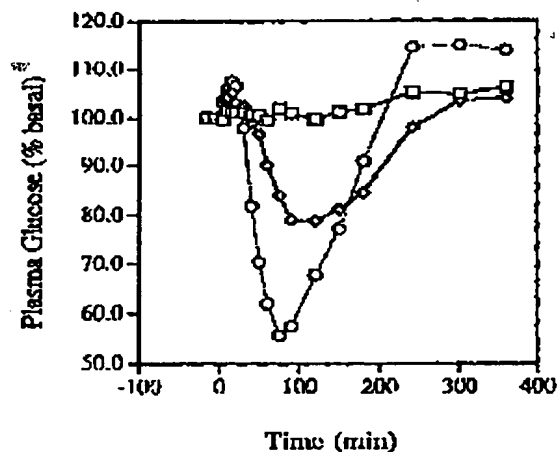
Priority Number(s): Jan. 14, 1997 **GB1997000006241**

Legal Status:  [Show legal status actions](#)

Designated Countries: **AU, CA, GB, JP, NO, NZ, US, European patent: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE**

Abstract: There is provided a pharmaceutical composition for use in the improved uptake of therapeutic agents across mucosal surfaces which comprises a mixture of chitosan and a type A, cationic, gelatin, together with a therapeutic agent. The composition is preferably in the form of microparticles, such as microspheres.
[\[Show "fr" Abstract\]](#)

Representative Image:



—□— Gelatin
—◇— Gelatin/chitosan 9.6% G210
—○— Gelatin/chitosan 19.28% G210

[\[Show "fr" image\]](#)

Attorney, Agent, or **BASSETT, Richard;**

The microspheres may be prepared by spray drying, emulsification, solvent evaporation, precipitation or other methods known to a person skilled in the art. The active drug can be incorporated into the microspheres during their production or sorbed onto the microspheres after their production. The microspheres or powder can be partially cross-linked by glutaraldehyde, formaldehyde, benzyldianone, benzoquinone, tripolyphosphate or other cross-linking agents known to the person skilled in the art. The conditions for carrying out the cross-linking, such as the amount of cross-linking agent required, are determined by monitoring the zeta potential and adjusting the conditions until the required zeta potential is obtained.

The size of the cross-linked or solidified microspheres are 1-200 μm , more preferably 1-100 μm .

If desired, other materials may be included in the composition, for example absorption enhancers. Suitable absorption enhancers include phospholipids such as lysophosphatidylcholine, lysophosphatidylglycerol and generally those mentioned in WO 88/09163.

The term "pharmacologically active compound" includes drugs, genes (DNA) or gene constructs, vaccines and components thereof (for example isolated antigens or parts thereof) and monoclonal antibodies.

The compositions may be used with drugs selected from the following non-exclusive list: insulin, PTH (parathyroid hormone), PTH analogues, calcitonins (for example porcine, human, salmon, chicken or eel) and synthetic modifications thereof, enkephalins, LHRH (luteinising hormone releasing hormone) and analogues (nafarelin, buserelin, leuprolide, goserelin), glucagon, TRH (Thyrotrophine releasing hormone), Vasopressin, Desmopressin, growth hormone, heparins, GHRH (growth hormone releasing hormone), nifedipine, THF (thymic humoral factor), CGRP (calcitonin gene related peptide), atrial natriuretic peptide, metoclopramide, ergotamine, Pizotizin, vaccines (particularly AIDS vaccines, measles, rhinovirus Type 13 and respiratory syncytial virus, influenza vaccines, pertussis vaccines, meningococcal vaccines, tetanus vaccines, diphtheria vaccines, cholera vaccines, DNA vaccines), pentamidine and CCK (cholecystokinin).

Further drugs include: antibiotics and antimicrobial agents such as tetracycline hydrochloride, leucomycin, penicillin, penicillin derivatives, erythromycin, sulphathiazole and nitrofurazone; antimigraine compounds such as sumatriptan or other 5-HT₁ agonists; vasoconstrictors such as phenylephrine hydrochloride, tetrahydrozoline hydrochloride, naphazoline nitrate, oxymetazoline hydrochloride and tramazoline hydrochloride; cardiotonics such as digitalis and digoxin; vasodilators such as nitroglycerine and papaverine hydrochloride; bone metabolism controlling agents such as vitamin D and active vitamin D₃; sex hormones; hypotensives; sedatives; anti-tumor agents; steroidal anti-inflammatory agents such as hydrocortisone, prednisone, fluticasone, prednisolone, triamcinolone, triamcinolone acetonide, dexamethasone, betamethasone, beclomethasone and beclomethasone dipropionate; non-steroidal anti-inflammatory agents such as acetaminophen, aspirin, aminopyrine, phenylbutazone, mefenamic acid, ibuprofen diclofenac sodium, indomethacin, colchicine and probenecid; enzymatic anti-inflammatory agents such as chymotrypsin and bromelain seratiopeptidase; anti-histaminic agents such as diphenhydramine hydrochloride, chlorpheniramine maleate and clemastine; antitussive-expectorants such as codeine phosphate and isoproterenol hydrochloride; analgesics such as morphine and its polar metabolites such as morphine-6-glucuronides and morphine-3-sulphate; antiemetics such as metoclopramide, ondansetron, chlorpromazine; drugs for treatment of epilepsy such as Clonazepam; drugs for treatment of sleeping disorders such as melatonin; drugs for treatment of asthma such as salbutamol.

The compositions can be administered via the nasal route as a powder using a nasal powder device, via the vaginal route as a powder using a powder device, formulated into a vaginal suppository or pessary or vaginal tablet or vaginal gel and via the pulmonary route using a powder inhaler or metered dose inhaler, via the rectal route formulated into suppositories and via the small intestine or colonic route formulated in tablets or capsules. The compositions may gel on the mucosa at least to some extent and this may facilitate retention of the composition on the mucosa.

Further disclosed is a method of treating a human or other mammal by administering a composition as described above to a mucosal surface of that human or other mammal, for example the vagina, rectum, lungs, eye, colon or nasal cavity.

Preferred embodiments of the compositions and methods will be further understood from the following non-limiting examples.

drug as compared to when the microspheres including the active drug are administered without the material, wherein the absorption enhancing material is selected from the group consisting of phospholipids, chelating agents, mucolytics, peptide inhibitors, and surface active agents selected from the group consisting of bile salts, fatty acids, fatty acid salts, acylglycerols, tyloxapols, acylcarnitine, fusidates, and mixtures thereof.

2. The method of claim 1 wherein the microspheres comprise a material selected from the group consisting of gelatin, albumin and collagen.

3. The method of claim 1 wherein the microspheres comprise a material selected from the group consisting of starch and dextran.

4. The method of claim 1 wherein the drug is a biologically active polypeptide.

5. The method of claim 1 wherein the absorption enhancing material is a biological surfactant selected from the group consisting of bile salts, fatty acids, fatty acid salts, acylglycerols, tyloxapols, acylcarnitines, phospholipids, lysophosphatides, fusidates, and mixtures thereof.

6. The method of claim 1 wherein the absorption enhancing material is selected from the group consisting of cyclodextrins, enamines, malonates, salicylates, glycyrrhetinates, chitosans, and mixtures thereof.

7. The method of claim 5 wherein the absorption enhancing material is a lysophosphatide.

8. The method of claim 7 wherein the absorption enhancing material is a lysophosphatidylcholine.

9. The method of claim 1 wherein the active agent and microspheres form a drug delivery system that is administered intranasally.

Description

FIELD OF THE INVENTION

The present invention relates to drug delivery systems and more particularly to a system which enhances the uptake of active drug material, particularly high molecular weight materials, especially from the nasal cavity.

References will be made to technical papers and other disclosures within this field which are included for purposes of explanation.

European patent applications 023,359 and 122,023 describe a powdery pharmaceutical composition for application to the nasal mucosa and methods for administration thereof. The pharmaceutical composition allows polypeptides and derivatives thereof to be effectively absorbed through the nasal mucosa. Similarly U.S. Pat. No. 4,250,163 describes a method for administering a medicament to the nasal mucosa where the preferred composition has mucoadhesive properties. European Patent application 123,831 has described how the use of biocompatible water soluble amphiphilic steroids other than natural bile salts are capable of increasing drug permeability across body surfaces to include the nose. The German Patent 2620446 describes an aqueous insulin preparation for nasal application containing a penetration enhancer in the form of an amphoteric, anionic or nonionic surface active agents, saponin, bile salts or surfactin. European patent application 230,264 describes an aqueous nasal drug delivery system for vaccines containing a high molecular weight drug, a gelling agent (e.g. hydroxy-ethylcellulose) and in some cases other additives (e.g. surfactants, glycerol, polyethyleneglycol).

None of the above patents and applications describes the use of microspheres for nasal administration nor the combination of a microsphere and an enhancing agent or other adjuvants that would be expected to provide enhanced bioavailability.

A microsphere preparation for nasal delivery has been described in PCT/GB86/00721 however this refers to materials having ion exchange properties and for one specific drug sodium chromoglycate for local effect rather than delivery to the general circulation.

At the present time the nose is being proposed as an alternative route for the delivery of drugs that will act within the systemic circulation. Particular attention is being focused on products of biotechnology, namely the peptides and proteins. Other drugs that are being suggested are those that are poorly absorbed orally or are extensively metabolised either in the gastro-intestinal tract itself or are subjected to first pass metabolism in the liver.

Nasal delivery is considered to have promise for the following reasons.

1. The nose has a large surface area available for drug absorption due to the coverage of the epithelial surface by numerous microvilli.
2. The subepithelial layer is highly vascularized.
3. The venous blood from the nose passes directly into the systemic circulation and therefore avoids the loss of drug by first pass metabolism in the liver.

A wide variety of drugs has now been tested for bio-availability after administration via the nasal route. Some drugs appear to be absorbed effectively and show bioavailabilities comparable to the intravenous route. However, most drugs show a low bioavailability when administered intranasally, but there are exceptions. The natural steroid progesterone is largely ineffective when administered orally. When given by the nasal route it is absorbed effectively with a bioavailability similar to that for an intravenous injection; the peak concentration appearing after approximately 6 minutes. If progesterone is given via the oral route then published data suggest that the bioavailability is of the order of 1.2% as compared to IV administration (1). The second example is the beta-blocker propranolol. This drug is metabolised extensively in the liver and possibly in the gut wall when administered orally. When the drug is given intranasally in a simple solution, plasma levels identical to intravenous administration can be obtained (2).

Insulin, a drug that has been studied extensively for intranasal delivery, can be delivered across the nasal membrane but the absorption efficiency is normally about 1.2% of the administered dose. Absorption can be improved by the use of so-called absorption enhancers. For example in a study by Salzman insulin was administered in the presence of a surfactant, Laureth 9 (3). Not only was a clear dose response relationship obtained but also the peak level appeared rapidly. The potency of the intranasal insulin was approximately 1/10th that of intravenous administered insulin. Clearly if insulin can be delivered to patients in a safe and reliable way by nasal administration then such systems could have potential for administration with meals in type 1 diabetes.

Chien and Chang (4) have summarised the absorptive capacity of the nasal routes for a variety of drug substances. It will be noted that those materials of high molecular weight, i.e. peptides and proteins are normally poorly absorbed via the nasal route. Also is noted the fact that most of the compounds, both those with high and low absorption efficiencies, show peak plasma levels within approximately 30 minutes. Thus absorption, whatever its extent, appears to be rapid but does not last for a particularly long time. This indicates that the drug may either be removed from the site of absorption or, if sufficiently labile, is degraded before further absorption can occur.

Factors affecting systemic absorption of drugs from the nose

The rapid clearance of nasal sprays from the nose can probably be considered to be a major factor in influencing loss of drugs from potential absorption surfaces. In addition, for the case of peptides and proteins, enzymatic degradation of the drug and molecular size may also have a role in giving low bioavailabilities.

Most workers in the field of nasal delivery have attempted to overcome the problem of inefficient absorption of drugs by using absorption enhancers e.g. in the form of bile salts or surfactants to modify the properties of the nasal mucosa thereby enhancing uptake. A typical example is the investigation described by Hanson et al (5) on the nasal delivery of the peptide salmon calcitonin. Here she showed clearly that a significant increase in plasma calcitonin could occur when the drug was given in combination with a surfactant. Thus, without enhancer only trace amounts of calcitonin appeared in the plasma whereas with enhancer involved the AUC increased 10 fold. Similarly, the striking effect of increasing amounts of bile salt (sodium deoxycholate) on the absorption of insulin has been well described by Gordon and others (6).

Controlled release systems for the nose

Illum et al (7) have chosen microspheres made from materials that are known to swell in contact with water to form a gel-like layer with good bioadhesive properties. Thus, due to their adherence to the nasal mucosa they could well modify clearance. The materials selected included albumin, starch and the ion exchange material DEAE-Sephadex. the size of the microspheres has been of the order of 40-60 μm in diameter.

The clearance of labelled microspheres from the nose has been studied in human volunteers using the standard technique of gamma scintigraphy (7). The microspheres were labelled with technetium-99m and applied to the nose in powder form using a nasal insufflator. Liquid and powder formulations were used as controls. The position of the noses of the volunteers was held constant on the collimator of the gamma camera using a specially designed template. Scintiscans were obtained over a suitable time period and regions of interest were created around the site of deposition in the nasal cavity. The time-activity profiles showed clearly that the nasal spray and powder formulations are cleared quite rapidly (with a time for 50% clearance ($T_{\text{sub.50\%}}$) of 15 minutes). In contrast, the microsphere systems have a much longer clearance time. After 3 hours about 50% of the albumin and starch micro-spheres and 60% of the DEAE-Sephadex microspheres still remain at the site of application. The half-time of clearance from this initial deposition site for DEAE-Sephadex microspheres were calculated to be about 4 hours. At the present time we are exploring whether these microsphere systems will provide an enhancement of the bioavailability of selected drug substances to include peptides and proteins. We expect that a decreased clearance rate and the possible protection of labile drugs against enzymatic attack will significantly increase absorption efficiency.

In relation to controlled release systems and the nose it is interesting to note that Nagai and colleagues (8) have been able to increase the absorption of insulin after nasal application to dogs by using a gelling formulation. Insulin was mixed with a cellulosic material and Carbopol 934 (polyacrylic acid) and applied as a powder formulation. Similarly, Morimoto and colleagues (9) have used a nasal gel (once again polyacrylic acid) as a delivery system for insulin and calcitonin in rats. A significant decrease in plasma glucose levels obtained as compared to the normal formulation indicated an increase in the absorption efficiency.

A major problem in drug delivery is the effective absorption of high molecular weight materials such as proteins and peptides across biological membranes. Normally such molecules are not taken up by the body if administered to the gastrointestinal tract, to the buccal mucosa, to the rectal mucosa, the vaginal mucosa or given as an intranasal system.

As discussed above and by Chien and Chang (4) recent studies with insulin have demonstrated that the absorption of such a compound can be increased if it is given together with a so-called absorption enhancer. These absorption enhancing materials have included surfactants of the non-ionic type as well as various bile salt derivatives. An increased permeability of membranes in the presence of these types of surfactant materials is not unexpected, indeed the literature in the field of gastroenterology contains a wide range of such absorption promoters. (For a review see Davis et al (10). However, such materials may not be acceptable for the chronic administration of pharmacological agents because of their irritant effects on membranes. This includes not only the non-ionic variety of surface active agents but also bile salts and bile salt derivatives (e.g. fusidic acid).

It is an object of the present invention to provide a drug delivery system which enhances delivery of high molecular weight materials.

The present invention therefore provides a drug delivery system including an absorption-enhancing amount of non-liquid microsphere particles containing a non-toxic physiologically effective amount of active drug and including a material associated with each particle, which material has the property of increasing the bioavailability of the active drug across a mucosal membrane.

Preferably the particles are administered in the form of a powder by spraying and have bioadhesive properties.

The preferred materials for increasing the bioavailability of the drug are phospholipids and lysophosphatidyl compounds such as lysolecithin, lysophosphatidyl-ethanolamine, lysophosphatidylglycerol, lysophosphatidyl-serine, lysophosphatidic acid etc. Other phospholipid compounds soluble in water can be expected to demonstrate similar effects for example short chain phosphatidyl-glycerol and phosphatidylcholine. A suitable concentration is from 0.02 to 10%.

This material should not produce any problems in terms of chronic toxicity because in vivo the material should be non-irritant and/or rapidly metabolised to a normal cell constituent that does not have any significant irritant effect.

Embodiments of the present invention will now be described by way of example with reference to the accompanying drawings, in which:

FIG. 1 illustrates in graphical form the effect of the use of the bioavailability improving material on the uptake of a drug in a first experiment;

FIG. 2 illustrates in graphical form the effect of the use of the bioavailability improving material and the administration in the form of microspheres;

FIG. 3 illustrates in graphical form the effect of the use of the material in a rat study experiment;

FIGS. 4 and 5 respectively show plasma glucose levels for rabbits given intranasal doses of Zn and Na insulin;

FIG. 6 shows plasma glucose levels obtained for administration intranasally of insulin in different forms;

FIG. 7 shows corresponding curves for plasma insulin levels;

FIG. 8 shows data from rat experiments with hGH given intranasally;

FIG. 9 shows data from sheep experiments with hGH given intranasally;

FIG. 10 shows mean % plasma glucose following administration of insulin with glycodeoxycholate alone and with starch microspheres;

FIG. 11 shows the corresponding plasma levels for FIG. 10;

FIG. 12 shows the effect of administration of insulin together with acyl-carnitine and starch microspheres on plasma glucose;

FIG. 13 shows the effect of administration of insulin with lysophosphatidylcholine-myristoyl alone and with starch microspheres;

FIG. 14 shows the effect of administration of insulin with lysophosphatidylglycerol alone and with starch microspheres;

FIG. 15 shows the effect of administration of insulin with *chitosan* alone and with starch microspheres;

FIG. 16 shows the comparison of administration of insulin with starch microspheres alone and together with N-acetyl cysteine; and

FIG. 17 shows a similar comparison to FIG. 16 using aprotinin.

The drug to be administered to a mucosal surface in the gastrointestinal tract, the eye, genital tract or the nose or lung could be administered as a viscous solution, a suspension or a powder, together with a bioavailability improving material or more preferably it should be administered in the form of a colloidal particle comprising a microsphere system. The advantage of using bioadhesive microsphere systems for administration to the mucosal surface is that such systems should allow a longer period of contact, especially if the microspheres are slowly degrading. This is particularly true for the nasal administration of drugs contained in microspheres produced from natural materials such as albumin, gelatin and especially starch.

In the present invention phospholipids and lysophosphatides such as lysoleuthin are the preferred material to be added to the active drug to act as to increase the bioavailability of the drug. Lysophosphatides are produced by the hydrolysis of phospholipids. Such materials are surface active and form micellar structures.

Lysophosphatidylcholine changes the permeability of membranes and allows the increased uptake of proteins and peptides including; for example, insulin, human growth hormone and other products of biotechnology and recombinant DNA methodologies. After administration the lysophosphatides are converted by the cells of the endothelial lining of the mucosa to the intact phosphatides which are normal cell components (see de Vries et al (11). (Lysolecithin itself is also present in cell membranes in very small quantities (12)). This rapid and efficient conversion of lysophosphatides into the complete phosphatide structure leads to much reduced adverse reactions and side effects in terms of irritation and toxicity.

A preferred material is the material lysophosphatidylcholine produced from egg or soy lecithin. Other lysophosphatidylcholines that have different acyl groups as well as lyso compounds produced from phosphatidylethanolamines, phosphatidylglycerols and phosphatidic acid which have similar membrane modifying properties may be used. Water soluble phospholipids with short acyl chains will also be appropriate since these are surface active. Acyl carnitines (e.g. Palmitoyl-DL Carnitine-chloride) are an alternative.

Other enhancing agents that are appropriate for use in the present invention include phosphatidylglycerols, lysophosphatidylglycerols, lysolecithins, chelating agents (EGTA, EDTA, alginates), surface active agents (especially non-ionic materials), acyl glycerols, fatty acids and salts, tyloxapol, **chitosan**, cyclodextrins, glycyrrhetinates and biological detergents listed in the SIGMA Catalog, 1988, page 316-321. Also agents that modify the membrane fluidity and permeability would be appropriate such as Enamines (e.g. phenylalanine enamine of ethyllacetoacetate), Malonates (e.g. diethyleneoxymethylene malonate), Salicylates, Bile salts and analogues and fusidates. Suitable concentrations would be up to 10%.

The same concept of delivery of a drug incorporated into or onto a bioadhesive microsphere with an added pharmaceutical adjuvant would apply to systems that contained active drug and mucolytic agent, peptidase inhibitors or irrelevant polypeptide substrate singly or in combination. A suitably mucolytic would be thiol-containing compounds such as N-acetylcysteine and derivatives thereof. Peptide inhibitors include actinonin, amastatin, antipain, bestatin, chloroacetyl-HO-Leu-Ala-Gly-NH.sub.2, diprotonin A and B, ebelactone A and B, E-64, leupeptin, pepstatin A, phosphoramidon, H-Thr-(tBu)-Phe-Pro-OH, aprotinin, kallikrein, chymostatin, benzamidine, chymotrypsin, trypsin. Suitable concentrations would be from 0.01 to 5%.

The microspheres should be of a size between 10 and 100 microns and prepared from a biocompatible material that will gel in contact with the mucosal surface. Starch microspheres (cross linked if necessary) are a preferred material. Other microspheres include gelatin, casein, dextrans, alginate, ararose, albumin, collagen, **chitosan**, poly vinylacetate, hyaluronic acid esters and polylae-. Preparation of these microsphere systems is well described in the pharmaceutical literature (see for example Davis et al (13)). Emulsion and phase separation methods are both suitable. The final microspheres can be modified by chemical crosslinking using agents such as 2,3-butadione, 1,5-glutaraldehyde and sodium trimetaphosphate or heat treatment. For example, microspheres according to the invention were prepared as follows:

Preparation of starch microspheres

Starch microspheres were prepared by an emulsion technique as follows:

5 g potato starch were dissolved in 95 ml of water at about 90.degree. C. A second solution was prepared from 3 g of polyethylene glycol (M.sub.w =6000) and 47 ml of water. This solution was heated to about 70.degree. C., whereafter the warm starch solution was added while stirring, to form an emulsion. When the two-phase system had formed (with the starch solution as the inner phase) the mixture was allowed to cool to room temperature under continued stirring, wherewith the inner phase was converted to gel particles. The particles were filtered off at room temperature and slurried in 100 ml of ethanol, whereafter the particles were again filtered off and laid to dry in air.

The yield was 90%.

Soluble potato starch microspheres was prepared by a coacervation technique as follows:

15 ml 5% starch solution (pH=7) was kept at a constant temperature of 70.degree. C. and stirred (500 rpm) while a 30% solution of polyethylene glycol was added (.about.7 ml) until phase separation had occurred, the system was stirred for further 15 min before it was cooled on ice during constant stirring. The microspheres were then isolated by filtration and freeze-dried. With a stirring speed of 500 rpm particles with a mean size of

33 μm \pm μm was produced.

Preparation of Albumin microspheres

Albumin microspheres were produced by an emulsification technique. 50 ml of highly purified olive oil was mixed with 75 ml of petroleum ether and prestirred for 5-10 min in a 125 ml beaker using a Heidolph mixer. To this mixture 0.4 ml of 25% w/v aqueous solution of rabbit serum albumin (RSA) in phosphate buffer (pH 7.4) was added dropwise and stirring was continued at 700 rpm for 15 min. The microspheres were stabilised by adding dropwise 0.1 ml of a 25% w/v glutaraldehyde solution under continual stirring for 15 min. The microspheres were isolated by centrifugation, washed with petroleum ether, filtered through a Millipore filter, washed again with petroleum ether and then ethanol and freeze-dried overnight.

The size of the microspheres was in the range of 40-60 μm .

Since the size of the microspheres was found to increase when drug was incorporated the manufacturing procedure was adjusted using a stirring speed of 900 rpm in order to obtain microspheres of the desired size range.

Albumin microspheres were prepared by a coacervation technique as follows:

10 ml 25% HSA solution (pH=5) was stirred (500 rpm) while a 30% solution of PEG was added (about 2.5 ml) until phase separation occurred. The system was stirred for 15 min before the albumin droplets were solidified by slowly heating the mixture to 90 degree C. and keeping it at this temperature for 30 min. Instead of heat denaturation, glutaraldehyde can be used to crosslink the albumin but this latter method seems to make the particles aggregate to a greater extent than that seen with the heat denaturation. The microspheres were then isolated by filtration and freeze-dried.

With a stirring speed of 500 rpm particles with a mean size of 43 μm \pm 6 μm was produced.

Preparation of Gelatin microspheres

Gelatin microspheres were prepared by an emulsion technique as follows:

100 ml olive oil (70 degree C.) was mixed with 10 ml 5-10% gelatin solution and the mixture was stirred at 500-1500 rpm keeping the temperature constant at 70 degree C., the emulsion is stirred for 15 min and was then cooled on ice during constant stirring. The microspheres were isolated by filtration, washed and freeze-dried.

A concentration of 10% gelatin and a stirring speed of 1000 rpm gives a mean particle size of 70 μm \pm μm .

Gelatin microspheres were prepared by a coacervation technique as follows:

30 ml 10% bovine gelatin (pH=8.5) was kept at a constant temperature of 50 degree C. and stirred (500 rpm) while a 30% solution of PEG was added (about 20 ml) until the coacervation region was reached. To control this step a nephelometer can be used. The mixture was cooled on ice during constant stirring. The microspheres were isolated by filtration and freeze-dried.

With a stirring speed of 500 rpm particles with a mean size of 60 μm \pm 10 μm was produced.

Preparation of *Chitosan* microspheres

Chitosan microspheres were prepared by an emulsion technique as follows:

Chitosan, as for example a glutamate salt (70% degree of deacetylation) was dissolved in water to a concentration of 5% w/v. 100 ml Soybean oil was mixed with 10 ml of the 5% *Chitosan* solution to form a water in oil emulsion. The microspheres were stabilized by adding dropwise 0.1 ml of a 25% w/v glutaraldehyde solution under continual stirring for 15 minutes. The microspheres were isolated by centrifugation, washed and freeze-dried. The size of the microspheres was in the range 10-90 μm .

The active agent can be incorporated into the microspheres during their formulation or sorbed into/onto the system after preparation. For example, rose bengal and sodium cromoglycate were used as model drugs for demonstration of incorporation into the microspheres.

Albumin microspheres were prepared as described above by an emulsion technique with a stirring speed of 900 rpm and the model drug dissolved in the albumin solution at various concentrations:

Concentrations of 0.5, 2, 4 and 5% w/v Rose bengal were used. Above 5% w/v the aqueous phase became too viscous and microspheres did not form. Due to the solution characteristics of sodium cromoglycate in water the highest concentration that could be used was 8% w/v. Thus, microspheres were manufactured from solutions containing 0.5, 1, 2 and 4% w/v sodium cromoglycate.

The maximum loading capacities for these compounds were found to be 170 .mu.g Rose bengal and 137 .mu.g sodium cromoglycate per mg albumin microspheres.

200 mg of starch microspheres (freeze-dried) were added to 4 ml of sodium cromoglycate solution (80 mg/ml) and left for about half an hour to swell. The microspheres were separated from the solution by centrifugation and washed with water to remove excess of drug and then freeze-dried.

The preparation of the starch-insulin system was carried out by adding the freeze dried starch microspheres to a phosphate buffer solution (pH=7.3) containing the insulin and the enhancer system, mixing for 1 hour and freeze drying until a light powder was obtained. A typical concentration of insulin and enhancer system (e.g. lysolecithin) would be 1 IU/mg/microsphere and 0.08 mg/mg microspheres, respectively. The micro-spheres can be loaded with both less or more drug and enhancer system. For example, starch microspheres in combination with insulin and lysophosphatidylcholine were made by preparing a solution of 107.13 mg insulin in 30 ml of water (3.571 mg/ml, 100 IU/ml. A 10 ml solution of the enhancer, lysophosphatidylcholine was prepared at 10 mg/ml in water. 10 ml of insulin solution were mixed with the enhancer solution and the required quantity of microspheres (1 g) were dispersed in the solution and the resultant suspension stirred for one hour at room temperature, and then freeze-dried to obtain the power formulation.

The freeze-drying were performed on an Edwards Modulyo freeze-dryer fitted with a bell-jar assembly and operated at a pressure of 0.08 torr, a condenser temperature of -53.degree. C. and a product shelf temperature of 20.degree. C. The freeze-drying process were allowed to proceed for 24 hours after which the final product were loaded into the administration devices and then stored with dessicant at 4.degree. C. for 16 hours prior to administration.

The effectiveness of the system can be controlled by the physical nature of the microsphere matrix and e.g. the extent of the crosslinking. The microsphere delivery systems could also include micro-spheres made from the active peptide or protein itself such as insulin microspheres.

Using the combination of microspheres and bioavailability increasing material, it has been found that the bioadhesive microsphere systems have the ability to greatly enhance the bioavailability of polar drugs when they are administered together with the system. This improvement is very much greater than the enhancement that can be achieved by the material itself. This potentiation of action is believed to be due to the greater retention of the delivery system in the nasal cavity. The concept has been shown to be successful for different drugs such as gentamicin, insulin, calcitonin, CCK, DDVAP and growth hormone. The material selected for these studies has been lysophosphatidylcholine (described above). The concept should work equally well with other materials (see list elsewhere) and with other drugs such as:

Insulin (hexameric/dimeric/monomeric forms)

Glucagon

Growth Hormone (Somatotropin)

Polypeptides or their derivatives (preferably with a molecular weight from 1000 to 300,000)

Calcitonins and synthetic modifications thereof

Enkephalins

Interferons (especially Alpha-2 Interferon for treatment of common colds)

LHRH and analogues (Nafarelin, Buserelin, Zolidex)

GHRH (Growth hormone releasing hormone)

Secretin

Nifedipin

Bradykin antagonists

GRF (Growth releasing factor)

THF

TRH (Thyrotropin releasing hormone)

ACTH analogues

IGF (Insulin like growth factors)

CGRP (Calcitonin gene related peptide)

Atrial Natriuretic Peptide

Vasopressin and analogues (DDAVP, Lypressin)

Antibiotics

Metoclopramide

Migraine treatment (Dihydroergotamine, Ergometrine, Ergotamine, Pizotizin)

Nasal Vaccines (Particularly AIDS vaccines)

FACTOR VIII

G-CSF (granulocyte-colony stimulating factor)

EPO (Erythropoietin)

Antibiotics and antimicrobial agents such as tetracycline hydrochloride, leucomycin, penicillin, penicillin derivatives and erythromycin, chemotherapeutic agents such as sulphathiazole and nitrofurazone; local anaesthetics such as benzocaine; vasoconstrictors such as phenylephrine hydrochloride, tetrahydrozoline hydrochloride, naphazoline nitrate, oxymetazoline hydrochloride and tramazoline hydrochloride; cardiotonics such as digitalis and digoxin; vasodilators such as nitroglycerin and papaverine hydrochloride; antiseptics such as chlorhexidine hydrochloride, hexylresorcinol, dequalinium chloride and ethacridine; enzymes such as lysozyme chloride, dextranase; bone metabolism controlling agents such as vitamin D.sub.3 and active vitamin D.sub.3 ; sex hormones; hypotensives; sedatives; and anti-tumor agents.

Steroidal anti-inflammatory agents such as hydrocortisone, prednisone, fluticasone, predonisolone, triamcinolone, triamcinolone acetone, dexamethasone, betamethasone, beclomethasone, and beclomethasone dipropionate; non-steroidal anti-inflammatory agents such as acetaminophen, aspirin, aminopyrine, phenylbutazone, mefenamic acid, ibuprofen, diclofenac sodium, indomethacin, colchicine, and probenecid; enzymatic anti-inflammatory agents such as chymotrypsin and bromelain seratiopeptidase; anti-histaminic

agents such as diphenhydramine hydrochloride, chlorpheniramine maleate and clemastine; anti-allergic agents (antitussive-expectorant antasthmatic agents such as sodium cromoglycate, codeine phosphate, and isoproterenol hydro-chloride).

Administration

The microspheres can be administered via the nasal route using a nasal insufflator device. Example of these are already employed for commercial powder systems intended for nasal application (e.g. Fisons Lomudal System). Details of other devices can be found in the pharmaceutical literature (see for example Bell, A. Intranasal Delivery devices, in Drug Delivery Devices Fundamentals and Applications, Tyle P. (ed), Dekker, New York, 1988).

The microspheres can be administered to the vagina in a freeze dried powder formulation. The microspheres are administered in a vaginal applicator and once in the vagina, the microspheres are released by pressing a syringe-type piston or similar release mechanism on the applicator. Once released, the microspheres will take up water and form a gel.

The microspheres can be administered to the eye in a gel formulation. Before administration, the microspheres could conveniently be contained in a two compartment unit dose container, one compartment containing the freeze-dried microsphere preparation and the other compartment containing normal saline. Prior to application, the two compartments are mixed and a gel is formed, which is then administered to the eye.

Animal nasal delivery studies

The following studies of nasal delivery in animal models (rats, rabbits and sheep) has been carried out in order to substantiate the invention.

Gentamicin:

The drug gentamicin was chosen as a model test substance. This polar compound is known to be poorly absorbed when administered into the nose (see for example Duchateau et al (17) and the biological availability can be enhanced by added bile salts.

Rat Studies:

The in situ rat model of Hirai et al (14) was used as modified by Fisher et al (15). Male Wistar rats of about 200 g were anaesthetized by intraperitoneal injection of 60 mg/kg of Pentobarbitone (Sagatal, 60 mg/ml). The rats were tracheotomized, the oesophagus sealed and the carotid artery cannulated.

A volume of the gentamicin solution containing 0.5% of the drug with and without added lysophosphatidylcholine (LPC) (0.2%) was instilled into the nasal cavity. Blood samples were withdrawn from the carotid artery at 0, 5, 10, 15, 30, 45, 60 and 120 min after drug administration. The gentamicin level was determined by the EMIT method (16). The effect of the LPC enhancer is demonstrated in FIG. 1. The administration of gentamicin solution alone resulted in a poor bioavailability whereas the adding of the enhancer system gave rise to a five fold greater peak level. The AUC's (from $t=0$ to $t=120$ min) were 128 and 557 $\mu\text{g min/ml}$, respectively.

Sheep studies:

Cross-bred (Suffolk and Texel) sheep were divided into groups of 3 and 2. The mean weight of the sheep was about 40 kg.

The animals were not fasted prior to the administration of gentamicin. An in-dwelling Viggo secalon universal central venous catheter of 1.2 mm, i.d. with a secalon universal flow-switch was placed in the right jugular vein of each animal on the first day of the study and whenever necessary was kept patent by flushing with heparinised normal saline (50 IU/ml). The catheter was removed upon completion of the study. For intranasal administration, the sheep were sedated by an IV dose of Ketamine hydrochloride at 2 mg/kg to prevent sneezing during administration. The sedation lasted about 3 minutes. The animals that received gentamicin by the IV route were also sedated.

For the intranasal administration of solutions, a blue-line umbilical cannula of 35 cm length (size 6 FG) was inserted into the nostril of the sheep to a preset depth of 10 cm before the delivery of the solution from a 1 ml syringe. For intranasal administration of powdered formulations, a BOC endotracheal tube (red rubber, cuffed) of 6.5 mm was loaded with the powder formulation and then inserted into the nostril of the sheep to a preset depth of 6 cm before blowing the powder into the nasal cavity.

The first group of sheep (n=2) was given 0.25 ml of gentamicin solution (386 mg/ml) (5.0 mg/kg) into each nostril. The second group (n=3) received 0.25 ml of gentamicin solution (386 mg/ml) (5.0 mg/kg) containing 2 mg/ml LPC into each nostril. The third group (n=3) received 5.0 mg/kg gentamicin and 0.2 mg/kg LPC in combination with starch microspheres (1.9 mg gentamicin-/mg starch microspheres). The last group of sheep (n=3) was given 2 mg/kg gentamicin administered intravenously as a solution (40 mg/ml) through the jugular vein. Blood samples (2 ml) were collected through the jugular vein at 0, 8, 16, 24, 32, 45, 60, 90, 120, 180 and 240 min after drug administration. The serum was separated by centrifugation and the samples stored at -20.degree. C. awaiting analysis. No heparin was added to any of the samples. The gentamicin level was determined by the EMIT technique (16).

A dramatic effect is seen when the gentamicin plus enhancer are administered in the form of the starch microsphere formulation, the blood level peaking at 6.3 .mu.g/ml as compared to 0.4 .mu.g/ml for gentamicin solution. The combination of microspheres plus LPC enhancer provides a blood level-time profile that is very similar to that obtained when gentamicin is given intravenously (FIG. 2).

The fact that a sharp peak is obtained using a combination of drug with microspheres and bioavailability increasing material is very surprising. The studies of Illum et al (7) would suggest that to show clearance of bioadhesive microspheres from the nasal cavity would result in the absorption of drug from the nasal cavity at longer time periods. That is the plasma level--versus the profile should be flat for a controlled release perforation. This is not found; the increase in absorption occurs at the earlier time periods. It is therefore suggested that besides acting in a bioadhesive capacity the microspheres are affecting the tight junctions of the cells in the nasal mucosa thereby allowing greater drug uptake. This effect on tight junction integrity appears to occur during the gelling process, perhaps as a result of removal of water from the cells in the mucosa to the microspheres.

Accounting for the doses administered the bioavailability for the intranasally administered gentamicin in combination with the LPC and gelling microsphere system is 57.3% as compared to the gentamicin given by IV dose.

Insulin

In all animal studies the glucose plasma levels were analysed using the glucose oxidase method. The plasma insulin levels were determined for the rabbit and sheep experiments by means of radioimmune assay using a double-antibody technique.

Rat Studies:

The Hirai's in situ model (as modified by Fisher) was used to study the nasal absorption of insulin using non-diabetic male Wistar Rats of 150 g fasted overnight. The rats were anaesthetized with an i.p. injection of 0.25 ml of Pentobarbitone (60 mg/ml).

A 250 IU/ml solution of Zinc (Zn)-human insulin was prepared in buffer (1/75M Na.sub.2 HPO.sub.4) of pH 7.3. In some experiments 0.2% of LPC or for comparison it Glycodeoxycholate (GDC) were added to the preparation as absorption enhancers. The experiments were performed in replicate (n=4). 10 .mu.l was administered into the nasal cavity equivalent to 16.67 IU/kg (2.5 IU/rat). Blood samples (0.2 ml) were collected into 5 ml fluoride oxalate tubes at 10, 6 and 2 min pre-administration and at 5, 10, 20, 40, 60, 90, 120, 180, 240 and 300 min post-administration. The blood was replaced by saline administered through the jugular vein.

FIG. 3 shows the glucose levels for rats given intranasal doses of Zn-insulin solution, Zn-insulin solution in combination with 0.2% LPC or Zn-insulin solution in combination with 1% GDC. The results indicate that insulin given intranasally as a simple solution is not effective in lowering the plasma glucose level whereas the addition of a material such as LPC causes a fast and significant drop in measured plasma levels. The LPC in a

concentration of 0.2% can be seen to have a similar effect to 1% bile salt in this in situ model where the cilia clearance mechanism is impaired.

Rabbit studies:

Preparations of Zn-insulin (mainly hexamer form) or Na-insulin (mainly monomer/dimer forms) were administered nasally to rabbits either as free insulin or as a microsphere delivery system with lysophosphatidylcholine (LPC) as a bioavailability increasing material. The experiments were performed in replicate (n=4).

Non-fasted New Zealand White female rabbits of average weight 3.5 kg were used in this study.

A 40 IU/ml Zn- or Na-human insulin solution was prepared in buffer (1/75M Na.sub.2 HPO.sub.4) of pH 7.3-7.4. In some experiments 0.2% LPC was added.

A total of 200 .mu.l of the solution (100 .mu.l in each nostril) was administered intranasally equivalent to about 2.3 IU/kg using an Eppendorf pipette.

The rabbits were dosed s.c. with insulin at 0.8 IU/kg or 0.6 IU/kg from a 14 IU/ml or 10 IU/ml aqueous solution, respectively.

The dose of starch microspheres and insulin was fixed at 2.5 mg/kg and 2.5 IU/kg, respectively. The dose of LPC was 0.2 mg/kg. The average weight of the rabbits was 3.5 kg.

25 mg of microspheres were placed in a small glass vial and 250 .mu.l of a 100 IU/ml insulin solution (Na- or Zn-insulin) was added followed by the 2 mg LPC and 250 .mu.l of distilled water. The microspheres were then allowed to stand for 2 h at room temperature in contact with the insulin solution before freeze drying.

Approximately 15 mg of the freeze dried powder from each individual vial was filled into the applicator tubing, and this was stored in a dessicator until use.

The rabbits were administered the suggested dose into the nasal cavity without sedation. Each rabbit was held on its back during, and for 10 second after, the application to ensure the delivery of the powdered formulation. Blood samples of 200 .mu.l and 2 ml for glucose and insulin determination, respectively, were collected from the marginal ear vein at 10 and 5 min prior to the administration and at 5, 15, 30, 45, 60, 90, 120 and 180 min post-administration. For insulin analysis, the blood collected was mixed gently in 5 ml heparinised (Li Heparin) tubes. For glucose analysis, the blood collected was mixed gently in 5 ml fluoride oxalate tubes. The blood samples for glucose analysis were kept on crushed ice awaiting immediate analysis. The blood samples for insulin analysis were spun at 3000 rpm and the plasma collected was stored at -20.degree. C. awaiting analysis.

FIGS. 4 and 5 show the plasma glucose levels for rabbits given intranasal doses of Zn-insulin or Na-insulin, respectively as simple solutions, in simple solutions with 0.2% LPC added or in combination with starch microspheres and LPC. Also shown is the plasma glucose levels for rabbits injected s.c. with Zn-insulin or Na-insulin. The results show that for both types of insulin (hexameric and monomeric/dimeric form) the administration of the insulin in combination with the LPC the plasma glucose levels are lowered significantly compared to the simple insulin solutions. However, even more drastic decreases in plasma glucose levels are seen when the insulin is administered in combination with the microspheres and LPC. The shape of the plasma glucose curves for the latter systems are surprisingly similar to the ones obtained for the s.c. administration, although the doses are 2.5 IU/kg compared to 0.6 IU/kg for the s.c. dosing.

Sheep Studies

Zn-crystallized highly purified semisynthetic human insulin, each 1 mg of pure protein is equivalent to 28 IU insulin. Insulin solutions were prepared in 1/75M phosphate buffer (pH 7.3).

Fifteen cross-bred (Suffolk and Texel) sheep were used in this study. The animals were ear-tagged and weighed prior to the study:

The mean weight in kg of the sheep (.+-S.D.) was 35.9 (.+-2.7). The animals were not fasted prior to insulin

administration because it is difficult to achieve this in practice and because of the possibility of inducing insulin resistance in the animals. The latter term means that under such conditions the sheep blood glucose levels would not respond as readily to the insulin administered.

An in-dwelling Viggo secalon universal central venous catheter of 1.2 mm i.d. with a secalon universal flow-switch was placed in the right jugular vein of each animal on the first day of the study and whenever necessary, was kept patent by flushing it with heparinised normal saline (50IU/ml). This catheter was removed upon the completion of the study.

Preparation of insulin solutions and powders:

Insulin stock solutions were prepared in 1/75M phosphate buffer (pH 7.3). These were then used as liquid formulations for intravenous and intranasal administration, and also in the preparation of the lyophilised microsphere formulations. The latter were prepared by dispersing the required quantity of micro-spheres in the insulin solution (+any LPC), stirring for 1 hour at room temperature, and then freeze-drying to obtain the powder formulation.

Administration of insulin formulations

Insulin was administered at 0.1 IU/kg via the intravenous route, at 0.2 IU/kg via the subcutaneous route, and at 2 IU/kg via the nasal route. Three sheep were used in each experiment:

- (1) Intravenous administration of insulin as an aqueous solution prepared at 4 IU/ml : Sheep J, K, and L on 24/11/87.
- (2) Intranasal administration of an aqueous solution, prepared at 200 IU/ml : Sheep A, B, and C on 24/11/87.
- (3) Intranasal administration of an aqueous solution, prepared at 200 IU/ml in combination with 0.2% LPC (0.02 mg/kg) : Sheep D, E, and F on 24/11/87.
- (4) Intranasal administration of insulin in combination with starch microspheres (2.5 mg/kg) and LPC (0.20 mg/kg) as a lyophilised powder. To prepare the formulation 500 mg of Spherex were dispersed in 30 ml of 1/75M phosphate buffer (pH 7.3) containing 400 IU insulin and 40 mg LPC, mixed for 1 h, and then freeze-dried : Sheep M, N and O on 26/11/87.
- (5) Intranasal administration of starch microspheres (2.5 mg/kg) without insulin. To prepare the formulation, 500 mg of Spherex were dispersed in 30 ml of 1/75M phosphate buffer (pH 7.3) mixed for 1 h, and then freeze-dried: Sheep G, H, and I on 24/11/87.
- (6) Subcutaneous administration of insulin as an aqueous solution prepared at 4.2 IU/ml.

For intranasal administration of solutions, a blue-line umbilical cannula of 35 cm length (size 6FG, Portex Ltd., Hythe, Kent, England) was inserted into the nostril of the sheep to a preset depth of 10 cm before the delivery of the solution from a 1 ml syringe. For intranasal administration of powdered formulations, a BOC endotracheal tube (red rubber, cuffed) of 6.5 mm was loaded with the powder formulation and then inserted into the nostril of the sheep to a preset depth of 6 cm before blowing the powder into the nasal cavity.

For intranasal administration, the sheep were sedated by an i.v. dose of Ketamine hydrochloride at 2 mg/kg. This was intended as a counter-measure against the animal sneezing during administration. The anaesthesia lasts for about 3 minutes. The animals which received insulin by the i.v. route were also sedated to counter-act any possible effect of ketamine on the blood glucose or insulin levels measured.

Blood samples of 5 ml were collected onto crushed ice from the cannulated jugular vein of the sheep at 15 and 5 min prior to the insulin administration and at 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, and 240 min post-administration. Each blood sample was divided into two parts. For insulin analysis, the blood collected (2.5 ml) was mixed gently in 5 ml heparinised (Li Heparin) tubes. For glucose analysis, the blood collected (2.5 ml) was mixed gently in 5 ml fluoride oxalate tubes. All blood samples following withdrawal were maintained on crushed ice, awaiting centrifugation which was then performed at 4.degree. C. and 3000 rpm. The plasma collected was stored at -20.degree. C. awaiting insulin and glucose analysis (radioimmune assay for insulin).

FIG. 6 shows the plasma glucose levels obtained for the administration intranasally of a simple insulin solution, of blank starch microspheres, of insulin solution with added 0.2% LPC, insulin as a microsphere formulation in combination with LPC and the intravenous administration of insulin. FIG. 7 shows the corresponding curves for plasma insulin levels. As seen in the rat and rabbit studies insulin administered intranasally as a simple solution does not have a significant effect on the plasma glucose level and the amount of insulin being absorbed via this route is indeed very low. Adding the (LPC) to the formulation increases the amount of insulin appearing in the circulation and hence results in a somewhat lower plasma glucose level. The administration of the insulin in combination the starch microspheres and LPC results in a 693% increase in AUC of plasma insulin as compared to a simple nasal insulin solution. At the same time the peak insulin level is increased with 1040%. The sharp level peak appears at 15-20 min and decreases rapidly as for intravenous insulin. Considering the glucose levels obtained when administering the insulin-microsphere-enhancer system the shape of the plasma glucose profile is very similar to the one obtained for the intravenous insulin. The relative bioavailability for this system is about 25% as compared to a subcutaneous injection of insulin.

The fact that the bioadhesive microsphere systems can provide plasma profiles similar to those obtained after IV dosing is very surprising. The bioadhesive microspheres seem to affect not only clearance but also absorption.

Human growth hormone

For all experiments biosynthetic hGH was used. The plasma levels were analysed using a solid-phase 2-site sandwich-ELISA technique. Plasma was assayed in duplicate at a dilution of 1/10 against a standard solution of B-hGH (0.11-7.0 ng/ml) prepared in antigen incubation buffer and also prepared in the appropriate dilution of plasma.

Rat studies:

As before the experiments were performed using the rat in situ model described by Hirai and modified by Fisher.

Non-fasted male Wistar rats of about 200 g were divided into groups of 4 and anaesthetized using an i.p. injection of 0.35 ml of pentobarbitone (60 mg/ml).

Three different hGH preparations were administered to the rats namely a 10 mg/ml solution of hGH in potassium phosphate buffer (1/75M), pH=7.2, as before with the addition of 0.05% LPC and as before with the addition of 0.5% LPC.

20 μ l (1 mg/kg) of either of the three preparations were administered intranasally by means of a plastic tubing.

All experiments were performed in replicate. Blood samples, 20 drops were collected and kept on ice at times 0, 5, 10, 20, 30, 40, 50, 60, 90, 120, 180, 240 and 300 min after the application. The plasma was separated and stored at -20.degree. C. until analysis.

From FIG. 8 it can be seen that hGH given intranasally as a solution without an enhancer system is not absorbed at any significant degree via the nasal membrane. However with the addition of 0.5% LPC to the solution the resultant plasma level peaks are increased from about 3.5 ng/ml to about 57 ng/ml with a very significant increase in AUC. The addition of a very low concentration (0.05%) of LPC has apparently no effect on the absorption of hGH.

Sheep studies:

Twelve cross-bred (Suffolk and Texel) sheep were used in this study. The animals were ear-tagged and weighed prior to the study:

The mean weight in kg of the sheep (\pm S.D.) was 35.8 (\pm 3.0).

An in-dwelling Viggo secalon universal central venous catheter of 1.2 mm i.d. with a secalon universal flow-

switch was placed in the right jugular vein of each animal on the first day of the study and whenever necessary, was kept patent by flushing it with heparinised normal saline (25 IU/ml). This catheter was removed upon the completion of the study.

hGH was administered at 34.2 $\mu\text{g/kg}$ (0.1 IU/kg) via the subcutaneous route and at 307.5 $\mu\text{g/kg}$ (0.9 IU/kg) via the nasal route. Three sheep were used in each experiment:

- (1) Subcutaneous administration of hGH as an aqueous solution prepared at 1.37 mg/ml (4 IU/ml).
- (2) Intranasal administration of hGH as an aqueous solution prepared at 17.57 mg/ml (51.43 IU/ml). A sheep of 40 kg would thus receive 0.35 ml of the formulation in each nostril (0.70 ml total).
- (3) Intranasal administration of hGH in combination with starch microspheres (2.5 mg/kg) and LPC (0.20 mg/kg) as a lyophilised powder. To prepare the formulation, 500 mg of Spherex were dispersed in 30 ml of sterile distilled water containing 61.5 mg hGH (180 IU) and 40 mg LPC, mixed for 1 h, and then freeze-dried:

For intranasal administration of solutions, a blue-line umbilical cannular of 35 cm length was inserted into the nostril of the sheep to a preset depth of 10 cm before the delivery of the solution from a 1 ml syringe. For intranasal administration of powdered formulation, a BOC endotracheal tube (red rubber, cuffed) of 6.5 mm was loaded with the powder formulation and then inserted into the nostril of the sheep to a preset depth of 6 cm before blowing the powder into the nasal cavity.

For the intranasal studies, it is necessary to sedate the sheep by use of an i.v. dose of Ketamine hydrochloride at 2 mg/kg. This is intended as a counter-measure against the animal sneezing during administration. The anaesthesia lasts for about 3 minutes.

The animals which received hGH by the s/c route were also sedated to counteract any possible effect of ketamine on the blood hGH levels measured.

Blood samples of 2 ml were collected in heparinised (Li Heparin) tubes onto crushed ice from the cannulated jugular vein of the sheep prior to the hGH administration and at 10, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 240, and 300 min post-administration. The plasma collected by centrifugation (3000 rpm at 4.degree. C.) were stored at -20.degree. C. awaiting analysis by the ELISA technique.

FIG. 9 shows the obtained hGH levels for the intranasal administration of a simple hGH solution, the intranasal administration of hGH in combination with microspheres and LPC and the subcutaneous injection of hGH. It can be concluded from the results that the hGH administered intranasally as a simple solution is not absorbed by any significant extent. However, when the hGH is administered in combination with microspheres and the LPC enhancer system the hGH plasma level is increased considerably. Thus, the peak plasma level is increased from about 10 ng/ml to about 55 ng/ml. The bioavailability as compared to subcutaneous injection can be calculated to about 20%.

The shape of the peak obtained using the microsphere-enhancer combination with LGH is also worthy of comment. A sharp peak, reminiscent of an intravenous dose is obtained. The subcutaneous administration of hGH gives a flatter profile, that reflects the slow release of the drug from the injection site. Thus as stated before for gentamycin and insulin the microspheres are not acting in a controlled release manner but providing a pronounced and surprising level of synergy when combined with the enhancing agent.

Comparative studies using insulin together with various materials

A series of studies were carried out to determine the effect on the administration of insulin of various enhancers with and without microsphere particles. All of the studies were carried out in sheep and the insulin was administered intranasally either in solution or powder formulation. The preparation of the various insulin formulations and the method of administration and result taking were all as described earlier.

FIG. 10 shows the mean % plasma glucose levels following administration of insulin (INS). 2 IU/kg in combination with glycodeoxycholate (GDC) 0.08 mg/kg alone and with starch microspheres (SMS) 2.5 mg/kg and FIG. 11 shows the corresponding plasma insulin levels. It can be seen that the plasma glucose level is decreased considerably more by the administration of insulin with GDC and starch microspheres than by

administration of insulin in the GDC solution. The corresponding plasma insulin levels demonstrate the enhanced absorption of insulin when administered with the microsphere formulation of the invention.

In FIG. 12, the results of administration of insulin together with starch microspheres and acyl-carnitine (CAR) are shown.

FIG. 13 demonstrates the considerably increased effect on plasma glucose levels found with administration of insulin in combination with lysophosphatidylcholine-myristoyl (LPC-M) and starch microspheres over administrations with LPC-M alone in solution. Similar results were found using lysophosphatidylglycerol (LPG) instead of LPC-M, (FIG. 14) and with *chitosan* (CNS) (FIG. 15).

FIGS. 16 and 17 shows the results of a study to compare changes in plasma glucose concentrations following administration of powder formulations of insulin with starch microspheres alone with those following administration of insulin with starch microspheres and a bioavailability increasing material. FIG. 16 shows the results using N-acetyl cysteine (N-Ac Cyst) and FIG. 17 shows the results using aprotinin. In both cases, the decrease in plasma glucose and hence the increase in absorption of insulin was considerably increased by the presence of the material.

In the above studies, it is interesting to note that the absorption profile of insulin shows a sharp peak, which is somewhat surprising. A flat sustained curve may have been expected as explained above.

All of the materials used in the above studies were found to be of comparative effectiveness.

References

1. Hussain, A., Hirai, S. and Bawarshi, R., Nasal absorption of natural contraceptive steroids in rats--progesterone absorption, J. Pharm. Sci. 70, 1981, 466-467.
2. Hussain, A., Hirai, S. and Bawarshi, R., Nasal absorption of propranolol from different dosage forms by rats and dogs, J. Pharm. Sci. 69, 1980. 1411-1413.
3. Salzman, R., Manson, J. E., Griffing, C. T., Kimmerle, R., Ruderman, N., McCall, A., Stoltz, E. I., Mullin, C., Small, D., Armstrong, J. and Melly, J. S., Intranasal aerosolized insulin, N. Eng. J. Med. 312, 1985, 1078-1084.
4. Chien, Y. W., and Chang, S. F., Intranasal Dry Delivery for Systemic Medications CRC Critical Reviews in Therapeutic Drug Carrier Systems 4, 67 ug (1987).
5. Hanson, M., Gazdick, G., Cahill, J. and Augustine, M., Intranasal delivery of the peptide, salmon calcitonin, in S. S. Davis, L. Illum and E. Tomlinson: Advanced Delivery Systems for Peptides and Proteins, Plenum Press, London, 1986, pp. 233-242.
6. Gordon, G. F., Moses, A. C., Silver, R. D., Flier, G. F. and Carey, E., Nasal absorption of insulin: enhancement by hydrophobic bile salts, Proc. Natl. Acad. Sci. U.S.A. 82, 1985, 7419-7423.
7. Illum, L., Jorgenson, H., Bisgaard, H., Krogsgaard, O., and Rossing N., Bioadhesive microspheres as a potential nasal drug delivery system. Int. J. Pharmaceut. 39, 189-199 (1987).
8. Nagai, T., Nishimoto, Y., Nambu, N., Suzuki, Y. and Sekine, K., Powder dosage form of insulin for nasal administration, J. Control, Rel. 1, 1984, 15-22.
9. Morimoto, K., Morisaka, K. and Kamada, A., Enhancement of nasal absorption of insulin and calcitonin using polyacrylic acid gel, J. Pharm. Pharmacol. 37, 1985, 135-136.
10. S. S. Davis, L. Illum and E. Tomlinson (Eds). Delivery systems for peptide drugs, Plenum, New York, 1987.
11. de Vries, A. C. J., Batenburg, F. F. and van Golde, L. M. G. Lysophosphatidylcholine acyltransferase and lysophosphatidylcholine:lysophosphatidylcholine acyltransferase in alveolar type II cells from fetal rat lung.

Biochem. Biophys. Acta 833 (1985) 93-99.

12. Christiansen, K. and Carlsen, J, Reconstitution of a protein into lipid vesicles using natural detergents. Biochim. Biophys. Acta 735 (1983) 225-233.

13. Davis S. S., Ilium, L. McVie, J. G. and Tomlinson E. (eds) Microspheres and Drug Therapy, Pharmaceutical, Immunological and Medical Aspects, Elsevier Science Publishers B. V., Amsterdam, 1983.

14. Fisher, A. N., Brown, K., Davis, S. S., Par, G. D. and Smith D. A., The effect of molecular size on the nasal absorption of water soluble compounds by the albino rat, J. Pharm. Pharmacol. 39, 1987, 357-362.

15. Hirai, S., Yashiki, T., Matsuzawa, T. and Mima, H., Absorption of drugs from the nasal mucosa of rat, Int. J. Pharm. 7, 1981, 317-325.

16. O'Connell, M. B., Hein, K., Halstenson, C. and Matzke, G. R. Heparin interference with tobramycin, netilmicin and gentamicin concentrations determined by EMIT. Drug Intell. Clin. Pharm. 18 (1984) 503-504.

17. Duchateau, G. S. M. J. E., Zuidema, F. and Merkus, W. H. M. Bile salts and intranasal drug absorption. Int. J. Pharm. 31 (1986) 193-199.